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SYNTHESIS OF ORGANIC DEUTERIUM COMPOUNDS

X. METHYL-d_3 IODIDE AND DEUTERIUM SUBSTITUTION PRODUCTS OF METHYL ACETATE[1]

BY B. NOLIN

ABSTRACT

Methyl-d_3 bromide was easily converted by treatment with calcium iodide into methyl-d_3 iodide in 94% yield. The latter was reacted on the 50-millimole scale with silver acetate or silver acetate-d_3 to yield methyl-d_3 acetate or methyl-d_3 acetate-d_3. Methyl iodide and silver acetate-d_3 gave methyl acetate-d_3.

INTRODUCTION

This paper deals with the synthesis of methyl acetate derivatives deuterated specifically in each of the two methyl groups and in both; the preparation of methyl-d_3 iodide required as an intermediate in a number of cases is also described. These esters have been prepared as part of a systematic investigation of the infrared absorption of acyl compounds (3) and their spectra will be discussed in a subsequent publication.

The procedure adopted for the synthesis of the methyl acetates was the reaction between the silver salt of a carboxylic acid and an alkyl iodide, which had previously proved satisfactory for the preparation of a number of deuterated ethyl acetates on the 10-25 millimole scale (2). The desired esters were thus prepared according to the following scheme:

$$RCOOAg + RI \rightarrow RCOOR + AgI,$$

where R = CH_3 or CD_3.

Silver acetate-d_3 was prepared as previously described (4). Methyl-d_3 iodide was obtained from the corresponding bromide (4) after prolonged and repeated treatment with calcium iodide hexahydrate. Preparative details are given in the experimental part.

EXPERIMENTAL*

Methyl-d_3 Iodide

Methyl-d_3 bromide (9.1 gm., 93 millimoles) was transferred through a vacuum line into a long necked 50-ml. flask containing calcium iodide hexa-

[1] *Manuscript received August 19, 1953.*
Contribution from the Division of Pure Chemistry, National Research Council, Ottawa, Ontario, Canada. Issued as N.R.C. No. 3120.
**All mass spectrometer analyses are corrected for C_{13}.*

hydrate (22.4 gm., 1.2 equivalents) and cooled in liquid nitrogen. The reaction vessel was sealed off and shaken at about 75°C. for a day. The volatile material was separated and treated with mercury and phosphoric anhydride to remove small amounts of free iodine and water. The vapor pressure at 0°C. was 169 mm. This indicated the presence of about 4% of unchanged methyl-d_3 bromide since the vapor pressures of deuterated methyl iodide and methyl bromide at 0°C. are 148 and 481 mm. respectively (1); mass analyses also showed an incomplete conversion at this stage. Weight, 13.0 gm.

The mixture of methyl-d_3 halides was treated with fresh calcium iodide for two days. The reaction material was worked up as above. Neither methyl-d_3 bromide nor other contaminants were detected in the product, which analyzed 97.8 mole % CD_3I and 2.2 mole % CD_2HI for a total isotopic content of 99.2 atom % deuterium. The vapor pressure at 0°C. was 147.2 mm. n_D^{20} 1.5263. Yield, 12.7 gm., 94%.

The conditions described above for the small-scale conversion of methyl bromide into methyl iodide are convenient, but not necessarily ideal. A larger excess of calcium iodide hexahydrate and a longer period of heating might eliminate the necessity of a second treatment. However, the use of anhydrous calcium iodide which was reported several times in the literature for the conversion of higher bromides is definitely unnecessary in the case of methyl bromide.

Deuterated Methyl Acetates

The iodide (35–55 millimoles) was transferred through a vacuum line into a 25-ml. flask containing the appropriate silver acetate* (1.1 equivalents) and cooled in liquid nitrogen. The flask was removed from the manifold, attached to a reflux condenser, and heated overnight at 65–75°C. The volatile material was separated on the vacuum line and reacted again with fresh silver acetate (0.3 equivalent). Usually no yellow coloration developed overnight during this second treatment and the reaction was then considered complete. In the preparation of methyl-d_3 acetate-d_3 a third treatment appeared desirable.

The ester was treated twice with small amounts of phosphoric anhydride, where some loss necessarily occurred. A small fraction was removed by allowing the material cooled in dry ice – acetone to distill under its own pressure into a receiver cooled in liquid nitrogen. A large middle fraction was then collected in a similar way. It had a practically constant vapor pressure at 0°C.; its refractive index did not differ from that of the removed fractions by more than ±0.0002 unit. The yield of pure product averaged 50%.

The refractive indices, vapor pressures, and deuterium contents of the three deuterated methyl acetates thus obtained are listed in Table I, together with those of the normal compound prepared by the same method. The isotopic purity of the deuterated esters corresponded to 99.0 atom % deuterium or more. The vapor pressure of normal methyl acetate is raised on complete deuteration by 1.6 mm. at 0°C. On the other hand, deuteration lowers the

**The isotopic purity of silver acetate-d_3 corresponded to 99.3 atom % deuterium.*

TABLE I

PHYSICAL PROPERTIES OF NORMAL AND DEUTERATED METHYL ACETATES

Compound	D atoms per molecule		Vapor pressure (mm., 0°C.)	n_D^{20}
	Found	Required		
CH_3COOCH_3	—	—	62.4*	1.3616**
CH_3COOCD_3	2.97	3	63.2	1.3607
CD_3COOCH_3	2.97	3	63.4	1.3603
CD_3COOCD_3	5.95	6	64.0	1.3595

*Literature (6), 62.1 mm.
**Literature (5), 1.36193.

refractive index by 0.0003–0.0004 unit per carbon–deuterium bond. However, the difference observed between the refractive indices of methyl-d_3 acetate and methyl acetate-d_3 may not be due entirely to experimental errors. Part of it could result in the last analysis from a smaller amplitude of the zero-point vibrations of the latter ester.

ACKNOWLEDGMENTS

The author wishes to thank Dr. L. C. Leitch who has initiated the present series of papers on the synthesis of organic deuterium compounds and in whose section this work has been done.

The mass spectrometer analyses were kindly performed by Miss F. Gauthier and Miss J. Fuller.

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SULPHUR ISOTOPE EFFECTS IN THE BISULPHITE ADDITION REACTION OF ALDEHYDES AND KETONES

I. EQUILIBRIUM EFFECT AND THE STRUCTURE OF THE ADDITION PRODUCT[1]

By W. A. Sheppard[2] and A. N. Bourns

ABSTRACT

Equilibrium constants for the isotopic exchange reaction between bisulphite ion and the addition product of eight aldehydes and ketones have been measured.

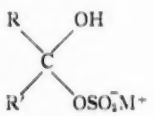
$$HS^{34}O_3^- + R_2CS^{32}O_3^- \rightleftarrows HS^{32}O_3^- + R_2CS^{34}O_3^-$$

(OH group on the addition product)

At 25° C., values ranging from 1.021 for acetone to 1.010 for anisaldehyde have been observed. Since constants of this magnitude are to be expected only if the bonding of the sulphur atom is different in the bisulphite ion and the addition product, these results are considered to confirm the carbon–sulphur bond structure of the latter. This conclusion is supported by the small constant, 1.002, obtained for the diethyl sulphate and ethyl hydrogen sulphate isotopic exchange in which the bonding of sulphur is essentially the same in the two reacting species.

INTRODUCTION

The problem of the structure of the bisulphite-addition product of aldehydes and ketones has occupied the attention of chemists for many years. By 1900, however, it had been fairly well established that these compounds are either α-hydroxysulphite ester salts, I, or α-hydroxysulphonic acid salts, II.



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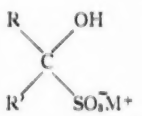

II

Structure I was originally favored on the basis of Müller's demonstration (8) that a product obtained by the action of sulphuric acid on methanol, and considered by him to be hydroxymethane sulphonic acid, was nonidentical with formaldehyde bisulphite. Raschig and Prahl (10,11,12), however, showed that Müller's product was symmetrical acetone disulphonic acid, formed by the sulphonation of acetone present in the methanol of that time, and, furthermore, that the actual product of the reaction of sulphuric acid and methanol is methyl hydrogen sulphate, an isomer of hydroxymethane sulphonic acid. These workers presented chemical evidence in favor of II, but their results were differently interpreted by Schroeter and Sulzbacher (13),

[1] *Manuscript received September 9, 1953.*
A contribution from the Department of Chemistry, Hamilton College, McMaster University, Hamilton, Ontario.
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who proposed a stable "trimolecular structure" represented by (R_2CO) (SO_2) (HOH). Backer and Mulder (1, 2), Lauer and Langkammerer (7), and Shriner and Land (14) have each provided strong support for structure II by converting the addition product to a compound considered to contain a carbon–sulphur bond by virtue of its preparation by known reactions from a sulphur compound of established structure. Although this evidence appears conclusive, there is the possibility of unexpected rearrangements, particularly in view of the lability of the carbon–sulphur bond (4).

Physical evidence in support of the hydroxysulphonic acid structure has also been reported. The absorption spectra of the formaldehyde and acetone addition compounds contain a band at 4992.0Å, compared to 4992.2Å found for sulphonic acids and 4996.0Å for metal alkali sulphites (15). Furthermore, Raman data (3), for the addition products of a number of carbonyl compounds suggest a carbon–sulphur bond and clearly eliminate the trimolecular formulation of Schroeter.

The present paper deals with a study of the fractionation of the sulphur isotopes in the exchange reaction between bisulphite ion and the addition product of a number of aldehydes and ketones (Equation 1).

$$\underset{(B^{34})}{HS^{34}O_3^-} + \underset{(P^{32})}{R_2C(OH)S^{32}O_3^-} \overset{K}{\rightleftarrows} \underset{(B^{32})}{HS^{32}O_3^-} + \underset{(P^{34})}{R_2C(OH)S^{34}O_3^-} \qquad [1]$$

The results provide what is considered to be a conclusive proof of the hydroxysulphonic acid structure, II, for the addition compounds.

The equilibrium constant, K, for this exchange reaction is given by:

$$K = \left(\frac{Q_{P^{34}}}{Q_{P^{32}}}\right) \Big/ \left(\frac{Q_{B^{34}}}{Q_{B^{32}}}\right) \qquad [2]$$

where the $Q_{P's}$ are the partition functions for the two isotopic species of addition compounds and the $Q_{B's}$ the corresponding partition functions for bisulphite ion (17). If the addition compounds have the carbon–oxygen–sulphur bond structure, I, the bonding of the sulphur atom is essentially the same as in bisulphite ion. Therefore the change in vibrational frequency of the sulphur bonds resulting from the substitution of S^{34} for S^{32} will be approximately the same in the two chemical species and the quotient of the two partition function ratios (Equation 2) will be very close to unity. If, on the other hand, the addition compounds contain a carbon–sulphur bond (structure II), the partition function ratios for the two chemical species will differ and an appreciable fractionation of the sulphur isotopes will occur. Indeed, this fractionation should be of the order of magnitude observed in the sulphur dioxide – bisulphite ion exchange reaction (Equation 3) in which the heavier isotope concentrates in the bisulphite ion to the extent of 1.9% at 25° C. (K = 1.019).

$$S^{34}O_2 + HS^{32}O_3^- \overset{K}{\rightleftarrows} S^{32}O_2 + HS^{34}O_3^- \qquad [3]$$

METHOD

The equilibrium constant for the exchange reaction between bisulphite ion and the addition compound may be expressed in terms of isotopic ratios as follows:

$$K = \frac{[B^{32}][P^{34}]}{[B^{34}][P^{32}]} = \frac{[B^{32}]}{[B^{34}]} \Big/ \frac{[P^{32}]}{[P^{34}]} \qquad [4]$$

where $[B^{32}]/[B^{34}]$ is the ratio of the sulphur isotopes in bisulphite ion at equilibrium and $[P^{32}]/[P^{34}]$ is the corresponding ratio in the addition compound. The equilibrium constant, K, was determined experimentally in the following way. The carbonyl component was shaken with excess saturated sodium bisulphite solution of natural isotopic abundance until equilibrium had been established, and the precipitated addition compound was collected and converted quantitatively to sulphur dioxide. Mass spectrometric measurement of the ratio of the sulphur isotopes in the latter gave the $[P^{32}]/[P^{34}]$ ratio in Equation 4. The ratio, $[B^{32}]/[B^{34}]$, could not be determined directly since the bisulphite ion at equilibrium could not be separated from dissolved addition compound. By using a large excess of sodium bisulphite, however, this ratio is practically identical with the isotopic ratio of the starting material. Furthermore, from a knowledge of the relative amounts of bisulphite ion and carbonyl component, the extent of conversion of the latter to addition product, and the isotopic ratio in the original bisulphite ion and the addition product, $[B^{32}]/[B^{34}]$ may be calculated with considerable accuracy.

RESULTS

In Table I are given the equilibrium constants, K, for the exchange reaction involving the addition product of three aldehydes and five ketones. Although isotopic equilibrium was normally attained within three days, several determinations were made for each compound using equilibration periods ranging from 5 to 24 days. Good agreement was found in every case.

The equilibrium constants differ from the ratios given in the second column from the right by an amount depending upon the sodium bisulphite: carbonyl compound ratio. The low solubility of the addition products of the higher molecular weight compounds permitted the use of a 10: 1 molar ratio, and the isotopic abundance of the bisulphite ion at equilibrium and that of the original bisulphite were therefore nearly the same. With acetone and 2-butanone, the greater solubility of the addition compound necessitated the use of only a small excess (two to three times) of bisulphite and the equilibrium constants are therefore considerably higher than the $\frac{(S^{32}/S^{34})\ \text{orig. } HSO_3^-}{(S^{32}/S^{34})\ \text{product}}$ ratios. Examination of the K values for 4-methyl-2-pentanone, however, shows that excellent agreement was obtained using widely different ratios of the reactants.

TABLE I

EQUILIBRIUM CONSTANTS FOR S^{32}, S^{34} EXCHANGE IN THE BISULPHITE ADDITION REACTION
(Temp. 25° C.)

Carbonyl component	Mol. wt.	$NaHSO_3$: carbonyl component (molar ratio)	Equil. time (days)	$S^{32}O_2/S^{34}O_2$*	$\frac{(S^{32}/S^{34})\text{ orig. bisulphite}}{(S^{32}/S^{34})\text{ prod.}}$	K
Acetone	58.1	1.9	24	22.776 ± 0.014**	1.010	1.021
		2.0	24	22.774 ± 0.015	1.010	1.020
Butanone	72.1	2.5	24	22.713 ± 0.013	1.013	1.022
		2.8	24	22.695 ± 0.015	1.014	1.021
4-Methyl-2-pentanone	100.2	4.7	5	22.678 ± 0.012	1.015	1.019
		8.0	24	22.626 ± 0.015	1.017	1.019
		10.0	24	22.631 ± 0.016	1.017	1.019
Benzaldehyde	106.2	10.0	7	22.748 ± 0.018	1.011	1.013
		10.0	25	22.731 ± 0.012	1.012	1.014
Heptanal	114.2	10.0	8	22.732 ± 0.014	1.012	1.014
		10.0	24	22.761 ± 0.017	1.011	1.013
2-Heptanone	114.2	10.0	14	22.757 ± 0.018	1.011	1.012
		10.0	24	22.765 ± 0.011	1.011	1.012
2-Octanone	128.3	8.0	24	22.746 ± 0.012	1.012	1.013
		10.0	24	22.739 ± 0.018	1.012	1.013
Anisaldehyde	136.1	10.0	17	22.799 ± 0.012	1.009	1.010
		10.0	24	22.799 ± 0.029	1.009	1.010

* *The $S^{32}O_2/S^{34}O_2$ ratios are relative to the $S^{32}O_2/S^{34}O_2$ ratio in the original $NaHSO_3$ arbitrarily chosen as 23.010.*

** *Precisions are expressed as standard deviation.*

The extent of conversion of the carbonyl component to the addition product was calculated as 100% for the three aldehydes and 99% for acetone and butanone, making use of the equilibrium constants reported by Gubareva (6). The 2-heptanone and 2-octanone addition products were actually isolated in a 98–99% yield, and it seemed reasonable to conclude that a comparable conversion was obtained with 4-methyl-2-pentanone, although the greater solubility of its addition product precluded isolation in high yield. It may be noted that an error of 20% in estimating the yield for the latter ketone introduces an error no greater than 0.1% in the exchange constant.

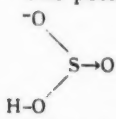
Validity of the Method and its Assumptions

In addition to the exchange reaction between bisulphite ion and the addition product, a second process which conceivably could contribute to the over-all fractionation observed is an exchange of the sulphur isotopes between solid and dissolved addition product. This effect, however, could only be significant in the case of the more soluble compounds. Furthermore, although the difference in solubility of the two isotopic species could not be measured for the addition products, it has been determined for sodium bisulphite itself. Solid sodium bisulphite was shaken with a saturated sodium bisulphite solution, the

solid and solution separated, and sulphur dioxide samples were prepared from each. Mass spectrometric analyses gave the following results:

Sample	64/66 mass abundance ratio
SO_2 from solid bisulphite	21.28 ± 0.02
SO_2 from bisulphite in solution	21.27 ± 0.02

It is apparent that there is no measurable fractionation of the sulphur isotopes between the two phases.

The possibility that bisulphite ion has the structure  rather than  must not be overlooked. If the ion contains a sulphur–hydrogen bond, then the argument, that fractionation of the sulphur isotopes would not be expected in an exchange with an addition product of the sulphite ester structure, might not be valid. However, the change in the bonding of sulphur in going from  to the sulphite ester form of the addition product corresponds to the change in going from this form of bisulphite ion to sulphite ion. If it therefore could be established that there is no fractionation in an exchange involving the two ions, it would seem reasonable to conclude that very little fractionation would occur in a bisulphite ion – sulphite ester addition product system.

The extent of fractionation between the two ions has been determined by taking advantage of the difference in water solubility of barium sulphite and barium bisulphite; the former is quite insoluble whereas the latter is readily soluble and, indeed, is known only in solution. A saturated solution of barium hydroxide was added to an excess of saturated sodium bisulphite and the mixture allowed to equilibrate. Sulphur dioxide samples, prepared from the precipitated barium sulphite and the solution, were analyzed mass spectrometrically with the following results:

Sample	64/66 mass abundance ratio
SO_2 from barium sulphite	20.43 ± 0.01
SO_2 from solution	20.43 ± 0.01

It is apparent that there is no measurable fractionation of the sulphur isotopes between the two ions. This result perhaps could be considered as evidence for the $HOSO_2^-$ structure of bisulphite ion.

Diethyl Sulphate—Ethyl Hydrogen Sulphate Isotopic Exchange Reaction

Further support for the assumption that no appreciable fractionation would occur between bisulphite ion and an addition product of the sulphite ester structure has been provided by a study of sulphur exchange in the equilibrium between diethyl sulphate and ethyl hydrogen sulphate (Equation 5).

$$C_2H_5OS^{34}O_2OH + C_2H_5OS^{32}O_2OC_2H_5 \rightleftarrows C_2H_5OS^{32}O_2OH + C_2H_5OS^{34}O_2OC_2H_5 \quad [5]$$

Although sulphur is in a higher oxidation state in the compounds involved in this exchange, nevertheless, assuming the $HOSO_2^-$ structure for bisulphite ion, the same bonds are being broken and formed as in a bisulphite ion – sulphite ester equilibrium. Diethyl sulphate was shaken with one-half mole equivalent of water for an extended period. The unconverted ester and ethyl hydrogen sulphate were separated and each converted to barium sulphate and thence to sulphur dioxide for mass spectrometric analysis. From the results given in Table II, it is seen that the equilibrium constant is only slightly greater than unity, confirming the prediction that isotopic fractionation is small in exchange reactions in which the bonding of the isotopic atom remains unchanged.

TABLE II

EQUILIBRIUM CONSTANTS FOR SULPHUR ISOTOPIC EXCHANGE IN THE DIETHYL SULPHATE REACTION

Temp., ° C.	S^{32}/S^{34} ratio*		Equilibrium constant
	Diethyl sulphate	Ethyl hydrogen sulphate	
25	22.095 ± .003	22.119 ± .003	1.001
55	22.080 ± .003	22.123 ± .003	1.002

* *Precisions are expressed as standard deviations.*

DISCUSSION

The equilibrium constants, given in Table I, for isotopic exchange in the eight bisulphite ion – addition product systems have values ranging from 1.010 to 1.021. These values are much larger than would be expected if the addition products were α-hydroxysulphite ester salts, and compare with equilibrium constants for exchange reactions in which the bonding of the isotopic atom is different in the chemical species concerned, e.g., the $SO_2 - HSO_3^-$ system. These results, therefore, are considered to provide a conclusive proof for the sulphonic acid salt structure of the carbonyl–bisulphite addition compounds.

The variation in the constants for the different carbonyl systems is somewhat greater than might have been expected. It is evident from Table I that this variation is in some degree related to the molecular weight of the carbonyl component. This, however, is not the only factor involved. It is seen, for example, that there is a very significant difference in the value of K obtained

for 4-methyl-2-pentanone and 2-heptanone but no such difference for 2-heptanone and 2-octanone, although the increment in molecular weight is the same in the two cases.

The present study is the first time, to our knowledge, that equilibrium constants for isotopic exchange reactions of this type have been determined with precision, and the results obtained suggest the value of further study of the effect of substituents on the magnitude of these constants.

EXPERIMENTAL

General

Sodium bisulphite of natural isotopic abundance was used in the investigation. This was possible since the abundance of S^{34} in nature is approximately 4% and, furthermore, it had the advantage over the use of enriched material in that any error of measurement arising from contamination of samples was smaller. Although the fractionation of S^{36} with respect to S^{32} is twice that of S^{34} to S^{32}, only the latter was determined because of the difficulties in the mass spectrometric measurement of the very low abundance of S^{36}.

Reagents

Sodium bisulphite (meta), Baker and Adamson powder, 95.9% assay.

Heptanal, Eastman Kodak White Label.

Benzaldehyde, Eastman Kodak White Label, was shaken with sodium carbonate solution and then with water, dried, and distilled *in vacuo*; b.p. 61–62° C. (10 mm.).

Anisaldehyde, Eastman Kodak White Label, was redistilled; b.p. 106.5–108° C. (5 mm.).

Acetone, Nichols N. F. Grade, was redistilled; b.p. 56° C.

Butanone, Eimer and Amend Technical Grade, was redistilled; b.p. 77–79° C.

4-Methyl-2-pentanone, Eastman Kodak Practical, was redistilled; b.p. 114.5–115° C.

2-Heptanone, Eastman Kodak White Label, was redistilled; b.p. 148–149.5° C.

2-Octanone, Eastman Kodak Practical, was redistilled; b.p. 169.8–170.9° C.

Diethyl sulphate, Eastman Kodak Practical, was redistilled; b.p. 99.5° C. (18 mm.).

Preparation of Bisulphite-Addition Compounds

To an aqueous solution of sodium bisulphite, containing 5.15 moles of bisulphite per liter of solution, was added the carbonyl component in the quantity necessary to give the desired bisulphite to carbonyl ratio. The reaction mixture, contained in a tightly-stoppered flask, was shaken on an agitating machine enclosed in a constant temperature bath maintained at 25 ± 0.5° C. The crystalline addition product was separated by suction filtration, washed with ethanol, and then with ether, and dried.

Preparation of Sulphur Dioxide Samples for Mass Spectrometric Analyses

The apparatus used for the preparation of sulphur dioxide samples is shown in Fig. 1. Solid addition compound, 6×10^{-4} moles, was placed in bulb *B* and

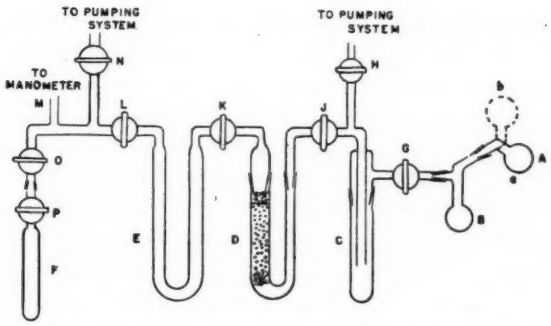


FIG. 1. Apparatus for preparation of sulphur dioxide samples.

0.5 ml. of 85% syrupy phosphoric acid dissolved in 0.5 ml. of distilled water in bulb *A*, which was turned to position "*a*". Both bulbs were surrounded with liquid air and the system evacuated. Stopcocks *H* and *J* were then closed and the liquid air replaced by hot water. Bulb *A* was turned to position "*b*" and the aqueous acid permitted to react with the addition compound. The reaction mixture was allowed to stand for two hours to equilibrate the oxygen isotopes of the sulphur dioxide and water, the latter being in 100-fold excess.* Following equilibration, liquid air was placed around trap *E*, a salt–ice mixture around *C*, and hot water (90–95° C.) around bulb *B*; stopcocks *G*, *J*, and *K* were opened and *L* and *H* closed. As the sulphur dioxide distilled into *E*, the water vapor largely condensed in *C* and was completely removed in the phosphorous pentoxide tower, *D*. To ensure complete recovery of sulphur dioxide, the water condensate was passed back and forth three times between *B* and *C*, using warm water and liquid air baths. Trap *E* was then surrounded by a dry ice – acetone bath, stopcock *N* opened, and carbon dioxide present as impurity removed by pumping on this trap a few seconds;** *N* was then closed. Sample tube *F* was surrounded by liquid air and, with stopcocks *L*, *P*, and *O* open, sulphur dioxide partially distilled into this tube.** Stopcock *L* was closed, *N* opened, and when a pressure of 10^{-6} mm. had been attained, *P* closed.

Samples of sodium bisulphite were decomposed to sulphur dioxide using a similar procedure. An aliquot of the saturated sodium bisulphite solution containing 6×10^{-4} moles of bisulphite (0.12 ml. of solution) was placed in bulb *B* and 0.5 ml. of phosphoric acid dissolved in 0.38 ml. of water used to effect decomposition.

Solid and Dissolved Sodium Bisulphite Equilibration

Twenty grams of sodium bisulphite was shaken with 40 ml. of saturated sodium bisulphite solution for seven days. The solid and liquid phases were

* *No data were available on the rate of oxygen isotope equilibration in this system. Analyses of sulphur dioxide samples prepared using 1.5, 2.0, and 2.5 hr. equilibration periods gave the same 64/66 mass ratios within the error of measurement. The equilibration time was standardized to 2.0 hr.*

** *It was established that no fractionation of the sulphur isotopes occurs in this process.*

separated and sulphur dioxide samples for mass spectrometric analysis prepared from each using the procedure already described.

Bisulphite–Sulphite Ion Equilibration

Fifteen milliliters of a saturated solution of barium hydroxide was shaken for five days with 30 ml. of saturated sodium bisulphite. The precipitated barium sulphite was filtered, washed with cold water, and dried. Sulphur dioxide samples were prepared from both solid and solution.

Diethyl Sulphate – Ethyl Hydrogen Sulphate Equilibration

Diethyl sulphate (0.04 moles) was shaken with 0.02 moles of water. The equilibration period was five weeks at room temperature in Run 1 and a similar period at this temperature followed by 75 hr. at 55° C. in Run 2. The unconverted ester and ethyl hydrogen sulphate were separated by partition between benzene and water. The aqueous solution, containing the ethyl hydrogen sulphate, was refluxed with 32 gm. of barium chloride and the precipitated barium sulphate removed periodically and ignited. The benzene solution was distilled *in vacuo* to remove solvent and the residual diethyl sulphate refluxed with 32 gm. of barium chloride in 100 ml. of water. Barium sulphate was filtered and ignited. Total recovery of sulphate was 97%.

Conversion of Barium Sulphate to Sulphur Dioxide

Barium sulphate was reduced with a mixture of hydroiodic, hydrochloric, and hypophosphorus acid to hydrogen sulphide (9), which was precipitated as cadmium sulphide from aqueous cadmium acetate and then converted to silver sulphide by addition of silver nitrate. The silver sulphide was oxidized to sulphur dioxide using a standard procedure (16).

Mass Spectrometric Analysis

In the bisulphite ion addition product equilibrium studies the relative isotopic abundance ratios were measured using both a 90° and 180° direction focusing Nier type mass spectrometer equipped with an automatic recorder (16, 5). The same analysis was obtained for a given sample with either instrument. The $S^{32}O_2/S^{34}O_2$ ratio was obtained from the ratio of mass 64 to mass 66 ion currents, the necessary correction being made for the contribution of the molecular species $S^{32}O^{16}O^{18}$ to mass 66. Each sulphur dioxide sample prepared from an addition compound was measured relative to a standard, this being the sulphur dioxide from the saturated sodium bisulphite solution to which was arbitrarily assigned an $S^{32}O_2/S^{34}O_2$ ratio of 23.010. The standard, the sample, and the standard were measured as quickly as possible in that order and the analysis was considered satisfactory if the two sets of results for the standard agreed within 0.1%, the precision of a single analysis. A single analysis consisted of six or seven spectrograms and each spectrogram consisted of masses 64 and 66, and then, by reversing the direction of scan, masses 66 and 64.

The relative abundance ratios for the sulphur dioxide prepared from diethyl sulphate and ethyl hydrogen sulphate were determined using the 90° instru-

ment equipped for simultaneous collection (18). This gave a precision of the order of 0.015%.

ACKNOWLEDGMENTS

We are indebted to Dr. H. G. Thode for helpful discussion, to Mr. E. R. Hayes, Mr. R. F. W. Bader, and Miss Midge Ishii for preparation of samples in the ethyl sulphate studies, and to Mr. R. K. Wanless and Mr. John Warren for mass spectrometer analysis of these samples. We also acknowledge financial assistance from the National Research Council which made this investigation possible.

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NEW COLOR REACTIONS OF SEDOHEPTULOSAN AND ITS DETERMINATION IN MIXTURES OF RIBOSE AND FRUCTOSE[1]

By L. Ujejski[2] and E. R. Waygood[3]

ABSTRACT

The reagents carbazole – sulphuric acid, cysteine hydrochloride – sulphuric acid, have been applied successfully to the quantitative colorimetric determination of sedoheptulosan in pure solution or in the presence of ribose and/or fructose. Fructose can be determined quantitatively in the presence of sedoheptulosan and/or ribose by using a combination of the two reagents. Results indicate that while sedoheptulose reacts differently to sedoheptulosan with the orcinol reagent, the reactions with carbazole and cysteine are not altered by hydration and these may form a basis for the determination of the naturally occurring seven carbon sugar sedoheptulose.

INTRODUCTION

The only color reaction reported for sedoheptulose to date is the formation of a blue color with orcinol (1, 9). Apparently this reaction is specific for ketoheptoses and the nonreducing anhydride sedoheptulosan only gives the blue color after hydration forming an equilibrium mixture of sedoheptulose and sedoheptulosan (1). Orcinol, however, has not proved to be satisfactory for the determination of carbohydrates in mixture (8).

On the other hand the reactions of carbohydrates with carbazole – sulphuric acid (3) and sulphydryl compounds especially cysteine hydrochloride (4, 6) have opened up new possibilities for their quantitative colorimetric determination. The former reagent has been applied successfully by many investigators (7, 8, 9, 10, 11) to the determination of various sugars and more recently Dische and Borenfreund (5) have used a combination of cysteine hydrochloride and carbazole – sulphuric acid for the determination of ketosugars and trioses.

This paper reports new colorimetric methods for the quantitative determination of sedoheptulosan based upon these reactions. The methods employed permit the determination of sedoheptulosan in pure solution or in the presence of ribose and/or fructose, also the determination of fructose in the presence of sedoheptulosan and/or ribose.

The authors are greatly indebted to Dr. Nelson K. Richtmyer (Federal Security Agency, Public Health Service, National Institute of Health, Bethesda, Md.) for a gift of three grams of sedoheptulosan. While this was sufficient to work out a method for its determination we were unable to obtain any samples of sedoheptulose to make a direct comparison of the methods for this sugar. However, determinations have been made with the equilibrium mixture of sedoheptulosan and sedoheptulose prepared by digestion of the former with 3 *N* hydrochloric acid at room temperature (12).

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Contribution from the Department of Botany, McGill University, Montreal, Canada. Acknowledgment is made to the Charles F. Kettering Foundation, Yellow Springs, Ohio, U.S.A., for a grant-in-aid.
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EXPERIMENTAL

Determination of Sedoheptulosan by the Carbazole – Sulphuric Acid Reagent

Twice sublimed carbazole was made up as a 0.5% solution in absolute alcohol. Sulphuric acid was refluxed with 20 mgm. of potassium persulphate per liter and diluted to 84% by weight. The reagent was prepared by making up 8.35 ml. of the carbazole solution to 250 ml. with 84% sulphuric acid. Nine milliliters of the reagent was placed in a test tube and cooled in an ice-water mixture for 10 min. One milliliter water (blank) or sugar solution was carefully layered above the reagent and the contents of the tube were then thoroughly mixed and heated in a boiling water bath for 13 min. During this period a red brown color appears. The tubes were then cooled for a further 10 min. in ice water and the intensity of the coloration measured in a Coleman Universal spectrophotometer (Model 14). Determinations were carried out on solutions containing 10, 20, 25, and 30 μgm. sedoheptulosan per ml. The absorption spectrum of the colored compound is shown in Fig. 1. The solution shows considerable absorption between 400 mμ and 600 mμ with a maximum at 490 mμ.

Over this range of concentration the colored compound obeys Beer's law and it was found that the ratio D490/D400 is characteristically constant (Table I). Equilibrium mixtures of sedoheptulose and sedoheptulosan prepared

TABLE I
OPTICAL DENSITIES AT 400 mμ AND 490 mμ OF SEDOHEPTULOSAN SOLUTIONS WITH CARBAZOLE – SULPHURIC ACID

Concentration, μgm. per ml.	D400	D490	D490/D400
10	0.075	0.142	1.89
20	0.142	0.270	1.90
25	0.176	0.340	1.93
30	0.225	0.424	1.88

by hydration of the latter give a similar colored compound with carbazole. The ratio D490/D400 does not change with hydration indicating that both substances react in essentially the same manner. This treatment, however, does result in a positive reaction with the orcinol reagent which indicates the presence of sedoheptulose.

Both ribose and fructose interfere with the determination and make it unsuitable for the determination of sedoheptulosan unless in pure solution.

Determination of Sedoheptulosan in the Presence of Ribose and/or Fructose

The procedure follows essentially the method of Dische (6) for the determination of hexoses. To 0.9 ml. of a solution containing sedoheptulosan alone or in the presence of ribose and/or fructose (for concentrations see Table II) 0.1 ml. of a 3% solution of cysteine hydrochloride and 5 ml. 38.4% sulphuric acid are added. The mixture is heated for 10 min. in a boiling water bath and then cooled in tap water and left at room temperature for 48 hr.

TABLE II
OPTICAL DENSITIES AT 510 mμ OF SEDOHEPTULOSAN SOLUTIONS WITH CYSTEINE HYDROCHLORIDE

Solution	Concentration, μgm. per ml.	D510
a. Sedoheptulosan	20	0.238
b. Sedoheptulosan	40	0.490
c. Sedoheptulosan	60	0.730
d. Sedoheptulosan Ribose	20 10	0.240
e. Sedoheptulosan Ribose	20 20	0.242
f. Sedoheptulosan Fructose	20 10	0.248
g. Sedoheptulosan Fructose	20 20	0.285
h. Sedoheptulosan Ribose Fructose	20 10 10	0.280

Sedoheptulosan gives a yellow orange color appearing at the end of the heating period and becoming more intense after 24–48 hr. changing color to red rose. Ribose under these conditions does not show any color, fructose a slight yellow at the beginning which eventually disappears. Sedoheptulosan shows a sharp absorption between 450 mμ and 550 mμ with a maximum at 500 mμ after one hour (Fig. 2). During the change in color from yellow-orange to red rose the absorption increases and the maximum shifts from 500 mμ to 510 mμ (Fig. 2). The optical densities at 510 mμ are shown in Table II for sedoheptulosan alone and in various mixtures. Table II shows that neither ribose nor fructose interfere with the observance of Beer's law. Furthermore, the optical density increment between 450 mμ and 550 mμ of the sedoheptulosan mixtures (*d*, *g*, *h*, Table II) has the same value as that for sedoheptulosan alone at 20 μgm. per ml. (*a*, Table II). Neither ribose nor fructose interfere at these concentrations. However, we have found that when fructose is raised above this level there is some interference.

After 48 hr. sedoheptulosan, ribose, and fructose all show considerable absorption in the ultraviolet between 270 mμ and 350 mμ with a maximum at 300 mμ for sedoheptulosan and ribose and 320 mμ for fructose (Fig. 3).

Determination of Fructose in the Presence of Sedoheptulosan and/or Ribose

To 1.0 ml. of a solution containing a mixture of the sugars, 0.2 ml. of a 1.5% solution of cysteine hydrochloride are added. Six milliliters 38.4% sulphuric acid was then added immediately followed by 0.2 ml. of 0.12% alcoholic solution of carbazole. The mixture was shaken and left standing at room temperature. The concentrations of the sugar solutions investigated are shown in Table III.

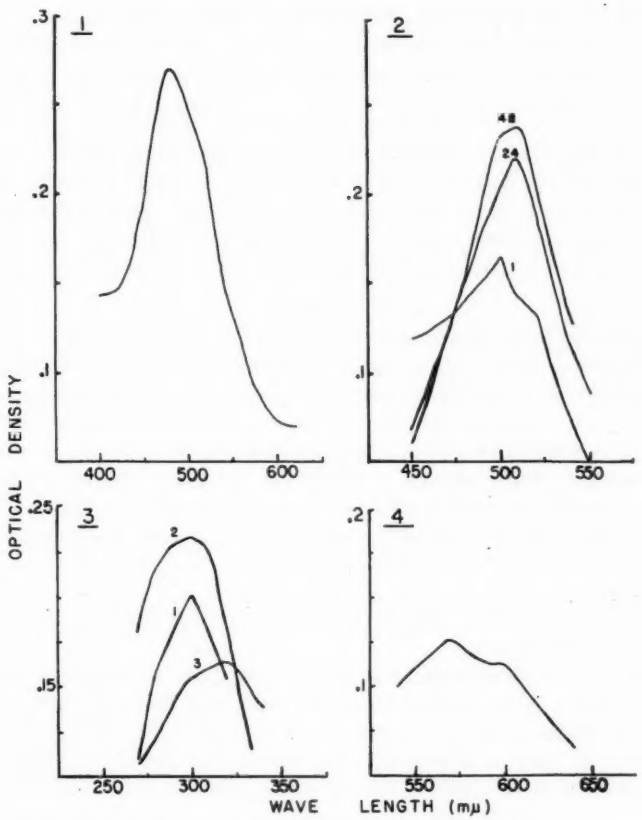

Fig. 1. Absorption spectrum of sedoheptulosan in carbazole reaction.
Fig. 2. Absorption spectrum of sedoheptulosan in cysteine reaction 1, 24, and 48 hr.
Fig. 3. Ultraviolet absorption spectrum of sedoheptulosan (1), ribose (2), and fructose (3) in cysteine reaction.
Fig. 4. Absorption spectrum of sedoheptulosan in cysteine–carbazole reaction after 24 hr.

TABLE III

OPTICAL DENSITIES AT 560 mμ OF FRUCTOSE SOLUTIONS WITH THE CYSTEINE–CARBAZOLE REAGENT

Solution	Concentration, μgm. per ml.	D560
Fructose	10	0.20
Fructose	20	0.41
Fructose	30	0.63
Fructose Sedoheptulosan	20 50	0.43
Fructose Sedoheptulosan Ribose	20 50 50	0.40

Solutions of sedoheptulosan and ribose whether alone or in a mixture produce a weak bluish color after one to two hours. On the other hand solutions of fructose alone or in mixture produce an intense purple color after a few minutes. Fructose absorption is maximum at 560 mμ. and neither sedoheptulosan nor ribose at a concentration of 100 μgm. per ml. interfere up to a period of one hour. After 24 hr. however, both the latter absorb strongly between 500 mμ and 700 mμ (Fig. 4). The determination of fructose in the presence of either or both of these two sugars is accordingly made after one hour duration. Table III shows that the optical densities of the fructose solutions obey Beer's law and there is no interference by either sedoheptulosan or ribose.

DISCUSSION

New color reactions of sedoheptulosan and fructose with carbazole – sulphuric acid and cysteine hydrochloride – sulphuric acid and a combination of the two reagents have been described for the first time. While sedoheptulosan can be determined in pure preparations by the use of the carbazole reagent the method is unsuitable for its determination in the presence of ribose or fructose owing to their interference. However, the use of the reagent cysteine hydrochloride has provided a method by which sedoheptulosan can be determined in the presence of either fructose or ribose. By using a combination of the two reagents fructose can be determined quantitatively in the presence of either sedoheptulosan or ribose. So far we have been unable to discover any colorimetric method by which ribose may be determined in the presence of these two other sugars.

In contrast to sedoheptulose, sedoheptulosan gives no color reaction with orcinol; however, there appears to be no difference between them in their reaction with either carbazole or cysteine. An equilibrium mixture of the two forms gave the same characteristically constant ratio D490/D400 = 1.90 with carbazole as would sedoheptulosan alone, and the same maximum with cysteine.

Owing to the increasing recognition given to the importance of the role played by sedoheptulose and its interrelation with ribose in plant metabolism (2) it was considered appropriate by the authors at this time to make known these new color reactions of sedoheptulosan. Further work on the microdetermination of sedoheptulose isolated from *Sedum spectabile* and *Bryophyllum calycinum* will be reported in a future publication.

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THE ACTION OF HYDROXYLAMINE ON METHYL ALPHA- AND BETA-D-GLUCOPYRANOSIDE TETRANITRATE IN PYRIDINE[1]

By L. D. Hayward[2] and C. B. Purves

ABSTRACT

The methyl glucoside tetranitrates reacted vigorously at room temperature with an excess of hydroxylamine base dissolved in anhydrous pyridine. Gas consisting of 91% nitrogen and amounting to 1.25 to 1.3 moles per mole was evolved within 20 min. and only a little more during the next 12 hr. Approximately 1.35 moles of nitrate groups in the original tetranitrate had been replaced by hydroxyl groups, for the most part at least without Walden inversions or other change, because hydrogenation of the sirupy product reduced it in more than 80% yield to crystalline methyl glucoside. The product from methyl-β-glucoside tetranitrate consisted of the 2,3,6-trinitrate (28%), the 3,6-dinitrate (17%), and an unidentified trinitrate (8%) which might have been a mixture.

The structures of the first two compounds were confirmed by preparing the fully methylated derivatives, denitrating the latter, and identifying the resulting known, partly methylated methyl-β-glucosides. New syntheses of methyl-β-glucoside-3,6-dinitrate, methyl 2,4-dimethyl-β-glucoside, and methyl-4-methyl-β-glucoside were found.

INTRODUCTION

Segall and Purves (38) found that 1 mole of nitrogen per glucose unit was evolved when cellulose trinitrate was dissolved in anhydrous pyridine containing an excess of hydroxylamine base, and approximately one mole of nitrate group was removed from the nitrocellulose. Apart from a small substitution (0.08) of oxime, the nitrate was replaced by a hydroxyl group which was probably of a secondary rather than a primary nature. Severe technical difficulties, however, caused further attempts to determine the structure of the partly denitrated cellulose to be deferred. It therefore became desirable to apply the hydroxylamine–pyridine reaction to the fully nitrated derivative of a simpler carbohydrate related to cellulose, and to locate the nitrate group or groups removed by the reagent. The present research employed the tetranitrates of methyl α- and β-glucopyranoside for this purpose, because data were available concerning their well-characterized, partly denitrated derivatives (13, 14, 15, 16, 35) which could also be converted, by methylation and subsequent denitration, to one or other of the known methyl ethers of methyl α- or β-glucoside (7, 13, 14, 15). Gladding and Purves (16) and Ansell, Honeyman, and Williams (4, 5) described the action of aqueous and alcoholic alkali on monosaccharide nitrates, but reports concerning organic bases seemed to be restricted to one by Wigner (40), who found in 1903 that pyridine alone removed one nitrate group selectively from mannitol hexanitrate. Hayward (18) recently showed that the group removed was in the third (or fourth) position.

Preliminary experiments on the stability of the methyl glucoside tetranitrates to anhydrous pyridine showed that, although highly colored substances

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Contribution from the Division of Industrial and Cellulose Chemistry, McGill University, Montreal, Que., and Pulp and Paper Research Institute of Canada, Montreal, Que.
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were formed, no gas was evolved at room temperature. About 60% of the unchanged tetranitrates could be recovered after 16 hr. by pouring the solutions into water. In a similar experiment with an alcohol solution of hydroxylamine, at least 75% of the β-tetranitrate could be recovered after 12 hr. Although gas was steadily evolved from the colorless solution, a blank containing no tetranitrate behaved in the same way and the observation was attributed to a slow decomposition of the base. The conjunction of either α- or β-tetranitrate with free hydroxylamine plus pyridine almost immediately produced a vigorous exothermic reaction; large volumes of a colorless gas were evolved, no unchanged tetranitrate could be isolated after 10 min., and the solution became only slightly yellow after 14 days.

Pure methyl-α-glucoside tetranitrate, which was more readily available than the β-isomer, was used to prepare a series of eight partly denitrated products isolated after reaction times extending from 10 min. to 12 hr. All these products proved to have refractive indices, methoxyl contents, and nitrogen contents that were practically the same. The partial denitration was therefore rapid, and the resulting pale yellow glass was stable or nearly so to the reagent. The fact that analyses for nitrate nitrogen were about 0.7% less than those for total nitrogen suggested that not all of the nitrogen was present as nitrate groups. By analogy with the behavior of cellulose trinitrate, the difference was tentatively attributed to an oxime substitution of about 0.18. On this basis the calculated average substitutions were methoxyl 0.85, nitrate 2.34, and oxime 0.18 moles per methyl glucoside unit, and the yield was about 80% of theory. Since the methoxyl substitution should obviously be unity, the above assumption was unsatisfactory, although no better one was found.

The partly denitrated methyl glucoside tetranitrate was submitted to catalytic hydrogenolysis over a palladized charcoal catalyst by Kuhn's method (26), and pure crystalline methyl-α-glucoside was recovered in a yield of only 82% of theory, whereas the recoveries from the original α- and β-tetranitrates were 95% to 96%. This decrease in yield provided another reason for the view that perhaps 15% of the product from the partial denitration had failed to retain the basic methyl glucoside structure. All attempts to isolate crystalline di- or tri-nitrates from the product failed, and the same lack of success was encountered when the methylated, benzoylated, or acetylated derivatives were examined, either before or after the removal of the remaining nitrate groups by hydrogenolysis. The α-series of products was therefore abandoned in favor of the β-series derived from methyl-β-glucoside, derivatives of which were often more readily crystallized (34). After two hours in the pyridine–hydroxylamine, the β-tetranitrate gave an 84% yield of a golden-yellow, extremely viscous sirup from which 90% of pure methyl-β-glucoside was recovered by hydrogenolysis.

The volume of gas evolved from the β-tetranitrate during the above reaction was measured at intervals, and was corrected for a rapid initial increase in temperature to about 70°C. The latter, nearly linear portion of the rate plot (Fig. 1), when extrapolated to zero time, corresponded to a production of

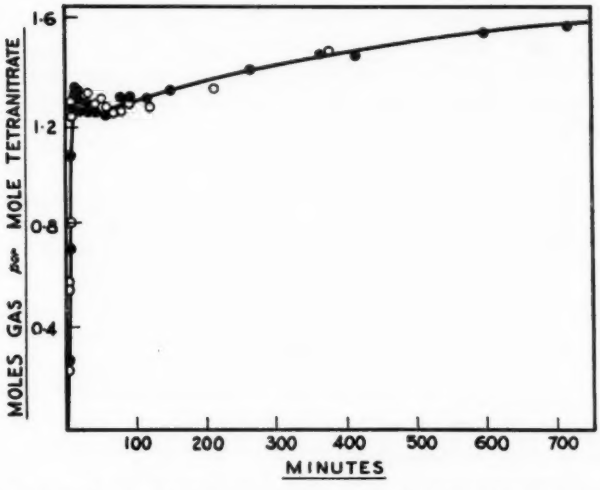


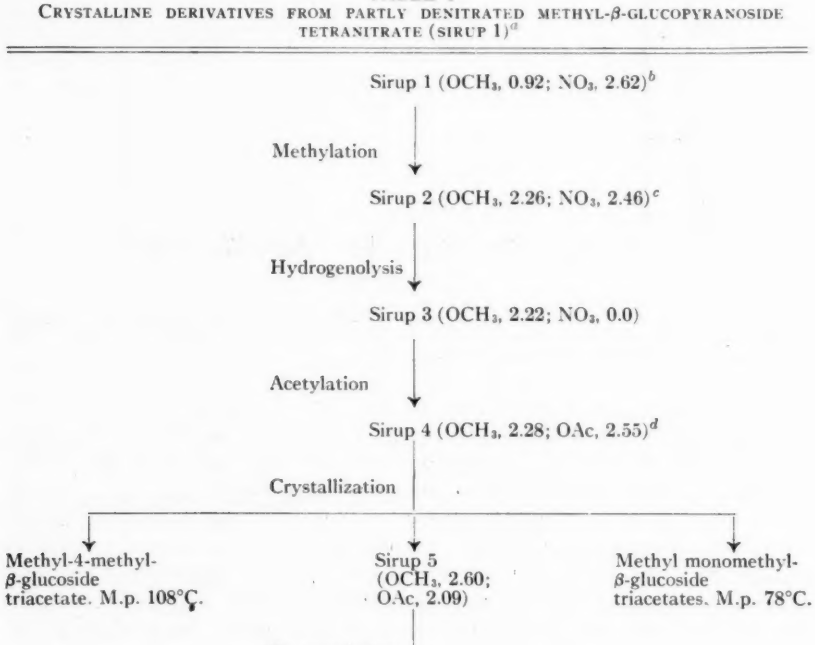
FIG. 1. The rate of gas evolution from 1 gm. of methyl-β-glucoside tetranitrate dissolved in 10 ml. of 12.5% hydroxylamine–pyridine. Corrected for temperature change and for the vapor pressure of pyridine (duplicate experiments).

about 1.25 moles per mole of tetranitrate in the initial rapid reaction. No good explanation was found for the slower reaction represented by the linear portion of the plot, because neither the yield nor the analyses of the partly denitrated product varied substantially with the reaction time, and because no gas was evolved in blank determinations with the pyridine–hydroxylamine reagent. Hydroxylamine, however, was known not to be stable in alkaline solution (30), and its breakdown might have been catalyzed by the sugar nitrate or its decomposition products, as well as by silver, mercury, and other ions (23, 33). The gas evolved during 12 hr. was shown by analysis in an Orsat apparatus and by its density to be nitrogen of 91.4% purity. An absorption of 8.6% in acid cuprous sulphate-β-naphthol solution was attributed to the pyridine vapor known to be present, rather than to carbon monoxide, and the solubility of nitrous oxide in pyridine made it possible that small amounts were formed but escaped detection. The presence of nitrous oxide was associated with oxime formation in the parallel research with cellulose trinitrate (38).

Five-gram portions of methyl-β-glucoside tetranitrate were then partially denitrated in pyridine–hydroxylamine for two hours with control of temperature (25° to 35°C.), and the products were combined, dried to constant weight, and identified as Sirup 1. If it was assumed that one-half of the gas evolved, in this case 1.3 moles per mole of tetranitrate, came from the hydroxylamine, the loss of the other half from the tetranitrate indicated that Sirup 1 should contain 12.0% of nitrogen and 9.84% of methoxyl groups. The values found were N, 11.8; OCH_3, 9.1%, the methoxyl content again being definitely low. Efforts were then directed to separating and identifying

the chemical constituents of the sirup by chromatographic methods, but the methods tried proved unsatisfactory because the literature yielded no information as to suitable conditions, and because reference compounds were inaccessible. Sirup 1 was then subjected to the series of reactions summarized in Table I.

TABLE I
CRYSTALLINE DERIVATIVES FROM PARTLY DENITRATED METHYL-β-GLUCOPYRANOSIDE TETRANITRATE (SIRUP 1)[a]



[a] *Five grams of the tetranitrate, 50 ml. of pyridine, and 6.25 gm. of hydroxylamine near 30°C. for two hours.*
[b] *Substitutions per glucose unit; nitrate substitution might include a little oxime.*
[c] *Substitutions per glucose unit.*
[d] *Calc. from yields and substitutions of the three components (next line of table).*

Methylation with silver oxide and methyl iodide gave Sirup 2 in practically quantitative yield and with little loss of nitrate groups. When these groups were removed by hydrogenolysis, the yield was again close to theory (Sirup 3) but was only 74% when this mixture of partly methylated methyl glucosides was acetylated with pyridine and acetic anhydride. The product, Sirup 4, crystallized in part, and systematic fractional recrystallization separated

crude methyl-4-methyl-β-glucopyranoside triacetate (48%), an isomeric triacetate (11%), and a very viscous, uncrystallized residue (Sirup 5). After purification, the identity of the 4-methyl glucopyranoside triacetate was unequivocally established by means of its melting point and specific rotation (8, 27, 31) and by de-acetylation to methyl 4-methyl-β-glucoside. This glucoside was obtained in the crystalline state, m.p. 101°C., in agreement with the recent independent work of McGilvray (29). The substance had the expected behavior toward aqueous sodium periodate, reducing 0.99 mole without the liberation of formic acid or formaldehyde. Acid hydrolysis yielded 4-methyl glucose (32) and its crystalline phenylosazone (13).

The melting point, 76° to 78°C., and the specific levorotation of −38° in chloroform found for the isomeric methyl monomethyl-β-glucoside triacetate did not agree with the values recorded for the 2-methyl (9), the 3-methyl (21), or the 6-methyl (19, 20) derivatives. De-acetylation resulted in a glass that could not be crystallized, either as such or as the benzoate. The glass had the methoxyl content of a methyl monomethyl hexoside, but when oxidized with aqueous sodium periodate consumed only 0.6 mole with the liberation of a little formic acid and no formaldehyde. These fractional amounts suggested that the glass was a mixture of various methyl monomethyl glucosides and that the homogeneity observed in the crystalline triacetate was spurious.

The residual Sirup 5 (Table I) was first de-acetylated with methanolic barium methylate, and the uncrystallized product did not change in specific rotation when dissolved for 48 hr. at 0°C. in anhydrous methanol–chloroform containing sodium methylate. Although de-acetylation appeared to be complete, a portion of the product could not be methylated with silver oxide and methyl iodide beyond OCH_3, 50.1, 49.6%, and no methyl tetramethyl β-glucoside (OCH_3, 62%) was recovered by fractional distillation of the methylated sample. It was assumed that Sirup 5 contained an acetyl group resistant to saponification by sodium or barium methylate, or of the type encountered by Helferich and Lang (21) and Dewar and Fort (13). The remainder of Sirup 5 was again submitted to de-acetylation, this time with aqueous sodium hydroxide (21), and 55% was recovered as crystalline methyl 2,4-dimethyl-β-glucoside with the correct melting point and rotation (13). The structure of this substance was confirmed by its failure to react with aqueous sodium periodate, by its hydrolysis to 2,4-dimethyl glucose, and by the capacity of the latter to yield the crystalline 4-methyl glucose phenylosazone (1,13) with elimination of a methyl group.

The above results clearly showed that the partial denitration of methyl-β-glucoside tetranitrate led to a mixture of nitrates which included the 2,3,6- and 3,6-derivatives. After standing for two months in a desiccator over phosphorus pentoxide, a sample of Sirup 1 (Table I) deposited in 5% yield the crystalline 3,6-dinitrate, which was identified by its rotation and melting point. This identity was confirmed by a quantitative methylation to the uncrystallized methyl 2,4-dimethyl-β-glucoside-3,6-dinitrate (13), and by hydrogenolysis of the latter in 93% yield to crystalline methyl 2,4-dimethyl-

β-glucoside (1, 13). Methyl-β-glucoside-3,6-dinitrate was more conveniently isolated by extracting a chloroform solution of Sirup 1 with water, in which the dinitrate was rather soluble (15). The yield was 11.9% by weight, but the extract also contained 20% of a yellow sirup that could not be crystallized. Evaporation of the chloroform solution left a sirup which on methylation yielded another with the approximate composition of a methyl monomethyl glucoside trinitrate. Found: OCH_3, 17.1, 17.5%. Calc. OCH_3, 18.1%. A series of experiments, analogous to those already described, recovered the 4-methyl, and the acetylated, unidentified monomethyl, derivatives of methyl glucoside from this trinitrate, but no other substances were identified. Sirup 1, after the partial removal of the 3,6-dinitrate by crystallization, was also acetylated, but fractional crystallization of the mixed acetate nitrates did not yield chemical individuals. These two series of experiments have not been described in detail because they merely confirmed, but did not extend, the results already reported.

Throughout the work noted in Table I and supported by other results, there was no indication that methylation produced an additional substitution of more than two in Sirup 1. The partial denitration of the methyl glucoside tetranitrate therefore appeared to remove only one or two nitrate groups from each molecule, and to give a mixture consisting exclusively of di- and tri-nitrates, perhaps contaminated with minor amounts of oximes and other substances. When this possibility was neglected, the production of tri- and di-nitrates was in a molar ratio of about 70:30 from the amount of gas evolved (Fig. 1), about 62:38 from the substitution of nitrate groups in Sirup 1 (Table I), and about 64:36 from the separation effected by extracting a chloroform solution of Sirup 1 with water. The 2,3,6-trinitrate and the 3,6-dinitrate preponderated, because about 28% of the original tetranitrate was recovered as crude methyl-4-methyl-β-glucoside triacetate, and about 18% as methyl-2,4-dimethyl β-glucoside, from Sirup 4. These yields might actually be greater, because losses of an undetermined nature reduced the yield of Sirup 4 to about 68% of theory.

The isolation of the unidentified methyl monomethyl glucoside triacetate, and of numerous intractable sirups with reasonable analyses, showed that the removal of nitrate groups from the tetranitrate by hydroxylamine in pyridine was to some extent of a random nature. Methyl-β-glucoside tetranitrate, moreover, failed to serve as a model of cellulose trinitrate, because the group most affected was in the fourth position of the former substance, and the fourth positions of the glucose residues of the latter were not nitrated. In both cases the partial denitration probably depended upon the superior reactivity of some nitrate groups, whose elimination stabilized the remainder. The elimination might involve the formation of nitrohydroxamic acid, NO_2NHOH, and its decomposition to nitrogen when in the presence of excess hydroxylamine (3), as previously discussed (38).

EXPERIMENTAL

Materials and Methods

Pure methyl-β-D-glucoside hemihydrate (24, 25, 28, or 36), when nitrated with acetic anhydride–acetic acid–100% nitric acid as described by Brissaud

(10), gave an 80 to 85% yield of the crystalline 2, 3, 4, 6-tetranitrate with the correct physical constants (6). Pure methyl-α-D-glucoside was nitrated with a phosphoric anhydride – 100% nitric acid mixture (38). The 2,3,4,6 tetranitrate, isolated in 86% yield, showed a marked tendency to remain as a supercooled liquid, but was recrystallized repeatedly by the slow cooling of its solution in ethanol. The analyses and physical constants (10, 26) of the product showed it to be quite pure. Since the two tetranitrates and also their partially nitrated derivatives were explosives, the scale of the preparations was restricted to 5 gm. or less, and all evaporations were under reduced pressure with bath temperatures below 50°C. Samples for analysis were dried to constant weight *in vacuo*. Vacuum distillation of the nitrates was not attempted.

Free crystalline hydroxylamine, prepared as required by a published method (38), was sensitive to moisture, heat, metals, and traces of alkali. The substance was immediately dissolved in pyridine which had been dried over barium oxide and which boiled at 115° to 115.5°C. The solution was stored at 0°C. for not more than 48 hr. before use.

Commercial electrolytic hydrogen and an Adams Low Pressure apparatus were employed in the hydrogenolyses. The palladized charcoal catalyst was prepared by the method of Hartung (17) and when thoroughly dry was pyrophoric. Two-gram-samples of methyl α- or β-glucoside tetranitrate, when hydrogenated in dioxane–ethanol at 30 to 40 p.s.i. and room temperature for 30 min. over 2 gm. of the catalyst, absorbed 7.6 and 7.8 moles of hydrogen per mole. Theory, 8 moles. The crystalline methyl glucosides were recovered from the filtered hydrogenated liquors in 96 to 98% yield, as Kuhn reported (26). Other hydrogenolyses of nitrate groups were carried out in the same manner.

Total nitrogen, nitrate nitrogen, and methoxyl groups were determined by methods previously indicated (38), and acetyl groups by saponification with alcoholic sodium hydroxide (11). In the oxidations with aqueous sodium periodate, the procedures described by several authors (2,22,37) were adapted and combined so that a single 100 mgm. to 200 mgm. sample revealed the change in optical rotation, the moles of periodate consumed, and of formic acid and formaldehyde produced.

Degrees of substitution were calculated by solving simultaneous equations of the type, $\%\ OCH_3 = 3100\ x/(180 + 14x + 45y + 13z + 42w)$, where x, y, z, and w were the molar substitutions of methoxyl, nitrate, oxime, and acetyl groups, respectively. One or more of the unknowns was usually zero in practice, and the small, doubtful oxime substitution z was usually ignored.

Action of Hydroxylamine–Pyridine on Methyl Glucoside Tetranitrates (Sirup 1)

In a typical experiment, 5 gm. of the β-tetranitrate was mixed with 50 ml. of an ice-cold 12.5% solution of pure hydroxylamine in pyridine contained in a flask fitted with a thermometer and calcium chloride drying tube. The nitrate dissolved readily to give a clear, colorless solution which almost immediately commenced to evolve gas, and to become hot. The final temperature was restricted to 25° to 35°C. by external cooling. After two hours, the solution, now pale yellow and still evolving gas, was poured into 500 ml.

of water, the aqueous mixture was rendered just acid to Congo red by adding 4 *N* sulphuric acid and was extracted with one 200 ml. and four 100 ml. volumes of ether. Continuous extraction of the aqueous residue with ether for seven days removed only 0.17 gm. of a brown liquid that soon changed to a black tar. The washed and dried ether extracts on evaporation gave the golden yellow glass denoted as Sirup 1 (Table I), which was very soluble in acetone, ether, and chloroform, and soluble in methanol, ethanol, and benzene. Found: OCH_3, 9.11, 9.19; total N (micro-Kjeldahl), 11.7, 11.9%. Calc. for glucose substituted with 0.92 methoxyl and 2.62 nitrate groups (mol. wt. 311): OCH_3, 9.18; total N, 11.8%. The average yield of 3.5 gm. was 84% of theory according to these substitutions.

One-gram samples of methyl-α-glucoside tetranitrate were separately dissolved in 10 ml. volumes of the pyridine–hydroxylamine reagent, but the solutions were precipitated into water after 10, 20, 30, 60, 120, 240, 360, and 720 min. at room temperature. The aqueous mixtures when extracted with chloroform gave uniform yields of pale yellow glasses with refractive indices within the range n_D^{20}, 1.4925 ± 0.0015, and of constant composition. Found for all samples: OCH_3, 8.72 ± 0.07; total N (micro-Kjeldahl) 11.65 ± 0.25%; for samples after 10 min. and 120 min., nitrate N (nitrometer) 10.93, 10.95%. Calc. for glucose substituted by 0.846 methoxyl, 2.34 nitrate, and 0.18 oxime groups (mol. wt. 300); OCH_3, 8.7; total N, 11.7; nitrate N, 10.09%. The yields, 0.65 gm., were about 80% of theory.

Gas Evolved from Methyl-β-glucoside Tetranitrate–Hydroxylamine–Pyridine

The tetranitrate, 1.00 gm., was placed in a 125 ml. round-bottomed flask fitted with a thermometer and dropping funnel, and connected to two 100 ml. gas burettes by a 3-way stopcock, substantially as described by Segall and Purves (38). A 12.5% solution of hydroxylamine in pyridine (10 ml.), was run into the flask and the volume and temperature of the gas evolved was noted at two to four minute intervals. The results (Fig. 1) were closely reproducible and were corrected for the vapor pressure of pyridine (31).

To determine the nature of the gas, samples were collected in the absence of air by carrying out the partial denitration for 12 hr. in the Toricellian vacuum of a Lunge nitrometer (38). The samples were then analyzed in an Orsat apparatus fitted with pipettes containing potassium hydroxide, potassium pyrogallate, and acidified cuprous sulphate-β-naphthol reagents (39) for the absorption of carbon dioxide, oxygen, and carbon monoxide, respectively. Found: CO_2, 0.0, 0.0; O_2, 0.0, 0.0; CO (apparent), 8.6, 8.6; residual gas, 91.4, 91.4%. The residual gas, when dried with anhydrous calcium chloride, was found to have a molecular weight of 28.8 by the method of Daniels, Mathews, and Williams (12). A control determination with commercial nitrogen of 98.4% purity gave the correct molecular weight of 28.02.

Methylation, Denitration, and Acetylation of Sirup 1 (Table I)

In a typical experiment, Sirup 1, 3 gm., dissolved in 75 ml. of methyl iodide, was heated under reflux with 10 gm. of silver oxide and 4 gm. of powdered Drierite for eight hours. Filtration and evaporation yielded 3.38 gm. of

Sirup 2, which gave a positive diphenylamine test for nitrate groups. Found: OCH_3, 21.8, 21.5; N, 10.7, 10.7%. Calc. for glucose substituted with 2.26 methoxyl and 2.46 nitrate groups (mol. wt. 322): OCH_3, 21.75; N, 10.7%. Remethylation did not alter the methoxyl content.

Hydrogenolysis of 6.78 gm. of Sirup 2 yielded 4.41 gm. (98%) of Sirup 3, a colorless, nitrate-free glass. Found: OCH_3, 32.5, 32.7%. Calc. for glucose substituted with 2.22 methoxyl groups (mol. wt. 211): OCH_3, 32.6%. A 7.15 gm. sample of Sirup 3 was acetylated overnight at room temperature with pyridine (196 ml.) and acetic anhydride (19.6 ml.) and 8.0 gm. (72%) of the acetate was recovered in a clear, colorless state (Sirup 4).

A solution of all of this product in 55 ml. of anhydrous ether was cooled to 0°C. and was diluted until just turbid with about 5 ml. of pentane. The long needles that separated were removed, and six further crops of crystals were recovered from the mother liquors over a period of four weeks. Evaporation of the mother liquors yielded 3.86 gm. of a tough, viscous sirup which was retained as Sirup 5. The first three crops of crystals, 3.06 gm., all melting between 105° and 108°C., and also the fourth, 0.17 gm., m.p. 96° to 99°C., were crude methyl-4-methyl-β-glucoside triacetate. The remaining three fractions, 0.88 gm., each melting rather sharply between 76° and 81°C., contained the crystalline isomer.

Methyl-4-methyl-β-glucopyranoside and its Triacetate

After recrystallization from ether, the crude triacetate, 3.23 gm., yielded 2.51 gm. of pure material as long, colorless needles, with the proper methoxyl content. The melting point, 107.5° to 108.5°C., and the specific rotation in chloroform, −34.8°, agreed closely with the published values (8,27,32).

A solution of 2.11 gm. in 50 ml. of anhydrous methanol was deacetylated according to Levene and Tipson (28) by adding 2.7 ml. of 0.2 *N* barium methylate in methanol. After standing overnight at 0°C., barium was removed as the carbonate, and the filtrate yielded 1.3 gm. of long needles. Repeated crystallization from ethyl acetate established the melting point of methyl-4-methyl-β-glucoside at 101° to 101.5°C., and the compound had specific levorotations of $[\alpha]_D^{20}$ − 21.0° in water (*c*, 2), − 17.4° in methanol (*c*, 3.44). McGilvray (29) found m.p. 102° to 103°C., and $[\alpha]_D^{20}$ − 17.6° in water.

A 200 mgm. sample of the glucoside was dissolved at 20°C. in water, 15 ml. of 0.1458 *M* sodium metaperiodate, $NaIO_4$, was added and the final volume was adjusted to 25 ml. The specific rotation changed from − 21° to − 126° in 80 min.; no formic acid or formaldehyde was formed and 0.99 moles of periodate per mole of glucoside was consumed.

Hydrolysis of the glucoside, 1.105 gm., with *N* sulphuric acid near 100°C. caused the specific rotation to assume the constant value of 51.0° in 11 hr., and 1.04 gm. of 4-methyl glucose was isolated as a colorless, copper reducing glass with the correct methoxyl content. A 3.846% solution of the sugar in water mutarotated from $[\alpha]_D^{20}$ + 62.1° (19 min.) to a constant value of + 56.2° (330 min.). Munro and Percival (32) reported the final value as + 53° (*c*, 2.1). The phenylosazone of the sugar was prepared in 58% yield, and after recrystal-

lization from aqueous acetone the fine yellow needles melted at 157.5° to 159°C., in agreement with the published value (32).

Methyl-monomethyl-hexoside Triacetate

Fractions 5 to 7 of Sirup 4, 0.88 gm., were recrystallized from alcohol-*n*-pentane until the melting point became constant at 76° to 77.5°C. and the rotation in chloroform was −34.6° (*c*, 16.4). Found: OCH_3, 18.7, 18.5; acetyl, 36.7, 37.7; N, 0.0, 0.0%. Calc. for $C_6H_7O_4(OCH_3)_2(CH_3CO)_3$: OCH_3, 18.6; acetyl, 38.6%.

An accumulation of 1.64 gm. of the triacetate was dissolved in 10.0 ml. of chloroform and was de-acetylated by adding 0.0165 gm. of sodium dissolved in 16.0 ml. of methanol to the solution. The observed rotation was $[\alpha]_D^{20}$ − 3.36° after four minutes and attained constancy at − 1.96° after 96 min. The product, 1.025 gm. (101%) could not be crystallized, and 4% solutions in water and methanol at 20°C. had specific rotations (D line) of − 22.1° and − 22.9°, respectively. Found: OCH_3, 28.3, 28.4%. Calc. for $C_8H_{16}O_6$: OCH_3: 29.8%.

A solution of 0.933 millimoles (0.194 gm.) of the monomethyl methyl hexoside in 25 ml. of 0.15 *M* sodium periodate changed in rotation from − 0.54° (3 min.) to constancy at − 1.14° (60 min.). No formaldehyde was detected in the liquor, but 0.012 millimoles of formic acid was produced and 0.556 millimoles (60%) of periodate was consumed.

Methyl-2,4-dimethyl-β-glucopyranoside and 2,4-Dimethyl Glucose

Sirup 5 (Table I), 3.725 gm., was de-acetylated with alcoholic barium methylate at 0°C. (28), and the uncrystallized product (Sirup 6), 2.95 gm., was dissolved in 40 ml. of water. When this solution was shaken with three 10 ml. volumes of chloroform, the portion extracted and the portion remaining in the aqueous residue had the same properties. Found in each: OCH_3, 32.9, 32.7%; n_D1.4668; $[\alpha]_D^{20}$ − 19.7° in water.

A second sample of Sirup 6, 0.55 gm., was again de-acetylated by solution for 20 hr. at room temperature in 15 ml. of 0.05 *N* sodium hydroxide (21). The alkali was exactly neutralized with sulphuric acid and the crystalline residue from the evaporation of the liquor was extracted with boiling acetone. The colorless crystals, 0.55 gm., recovered from the acetone melted at 99° to 113°C., and recrystallization from alcohol–petroleum ether gave 0.22 gm. of Sirup 9, which was not further examined, and 0.28 gm. of pure methyl-2,4-dimethyl-β-glucoside. Yield, 55% of theory from Sirup 5.

Pure methyl-2,4-dimethyl-β-glucoside, which could also be recrystallized from carbon tetrachloride, melted at 123.5° to 124°C. and had specific rotations of $[\alpha]_D^{20}$ − 17.7° in acetone (*c*, 3.3), and − 26.5° in water (*c*, 3.26). Dewar and Fort (13) found m.p. 124°C. and a rotation of − 16.3° in acetone; while the values quoted by Adams, Reeves, and Goebel (1) were m.p. 122° to 124°C. and $[\alpha]_D^{29}$ − 18.6°. A second m.p. of 107° to 108°C. was found for the freshly remelted substance, as Adams and his co-workers reported. No change in rotation or pH occurred when the glucoside was dissolved in aqueous sodium periodate at 20°C. for four hours. No periodate was consumed and no formaldehyde could be detected in the final solution.

A 0.81 gm. sample of the pure glucoside, when hydrolyzed with boiling *N* sulphuric acid, gave 0.77 gm. of 2,4-dimethyl glucose as a sirup that did not crystallize. Found: OCH_3, 29.3, 29.0%. Calc. for $C_6H_{10}O_6(OCH_3)_2$: OCH_3, 29.8%. The product readily reduced Fehling's solution, and its specific rotation in water changed from $[\alpha]_D^{20} + 59.2°$ (*c*, 2.88) after 11 min. to a constant value of 62.2° after two hours. When heated near 100°C. for 2.5 hr. with 10 ml. of water, 3 gm. of sodium acetate, 2 gm. of phenylhydrazine, and 3 drops of saturated sodium bisulphite solution, 0.72 gm. of the dimethyl glucose yielded a red oil that was extracted with ether. After being washed with 4 *N* acetic acid, cooling of the ether solution and the addition of *n*-pentane caused the crystallization of 0.05 gm. of 4-methyl glucose phenylosazone. Recrystallization from aqueous acetone caused the product to melt correctly at 157° to 158°C., and a mixed m.p. with an authentic sample was not depressed.

Methyl-β-glucopyranoside-3,6-dinitrate

This dinitrate was conveniently isolated by dissolving 26.5 gm. of Sirup 1 (Table I) in 500 ml. of chloroform and extracting the solution four times with 200 ml. volumes of water. The combined aqueous extracts were then continuously re-extracted with ether for 24 hr., or until a fresh ether extract gave no color in the diphenylamine test for the nitrate group. Evaporation of the ether then left 8.56 gm. of a semicrystalline mass which on recrystallization from chloroform gave 3.14 gm. (11.9% by weight) of the dinitrate.

The large colorless prisms of methyl-β-glucoside-3,6-dinitrate when recrystallized from cold chloroform melted at 144° to 145°C. and had a specific rotation of $[\alpha]_D^{20} - 7.2°$ in acetone (*c*, 1.52), in agreement with the observations of Dewar and Fort (13). Found: OCH_3, 10.9, 10.3; N, 9.85, 9.84%. Calc. for $C_6H_9O_5(OCH_3)(NO_2)_2$: OCH_3, 10.9; N, 9.86%.

Two grams of the pure dinitrate, when twice methylated with silver oxide and methyl iodide, yielded 2.15 gm. of an uncrystallized product with the methoxyl and nitrogen contents of methyl-2,4-dimethyl-β-glucoside-3,6-dinitrate. The refractive index, n_D^{20}, 1.4623, and the specific rotation in chloroform, $[\alpha]_D^{20} - 7.9°$, (*c*, 5.36) were close to those reported (13). A 1.34 gm. sample of this methylated dinitrate on hydrogenolysis gave 0.89 gm. (93%) of crystalline, nitrate-free methyl-2,4-dimethyl-β-glucoside with the proper rotation, m.p., and mixed m.p. (13).

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A NEW GENERAL SYNTHESIS OF 2-AMINO ALCOHOLS[1, 2]

By Louis Berlinguet[3]

RÉSUMÉ

Une nouvelle synthèse permet d'obtenir les 2-amino alcools à partir de l'acétamidomalonate d'éthyle. La méthode consiste à condenser un dérivé halogéné approprié avec le sel de sodium de l'acétamidomalonate d'éthyle. Par monosaponification de ce di-ester suivie de décarboxylation, on obtient un ester éthylique *N*-acétamidé qu'on réduit par l'hydrure de lithium et d'aluminium en alcool *N*-acétamidé. Par hydrolyse, on obtient le chlorhydrate du 2-amino alcool correspondant. Le D,L-phénylalaninol, le D,L-tyrosinol et le D,L-leucinol ont ainsi été préparés avec d'excellents rendements. La méthode semble être d'application générale et les produits intermédiaires sont faciles à isoler.

INTRODUCTION

2-Amino alcohols are an important class of compounds owing mainly to their pharmalogical and physiological properties (14). With the development of new techniques for the degradation of proteins (6, 9, 12), amino alcohols related to natural amino acids have assumed a new importance. In these stepwise degradations, reduction of an esterified protein at a free carboxyl end gives an amino alcohol which is separated from the amino acids and identified by a suitable analytical method (4, 8).

In order to investigate a new method for the determination of 2-amino alcohols, it became of interest to develop a new general synthesis that would allow these compounds to be prepared in substantial quantities and in analytically pure form. Some of these 2-amino alcohols have been prepared by the reduction of the corresponding natural amino acids or their derivatives. Karrer and his co-workers (15, 17) and Enz and Leuenberger (11) have reduced the ethyl esters of some amino acids with sodium in alcohol. Some years later, Karrer *et al.* (16) and Barrow and Ferguson (3) acetylated the amino acid esters, thus improving the yields in the reduction.

Some workers have tried catalytic reduction with hydrogen of the amino acid esters (7, 20, 21) and of the α-benzylamino acids (23), but in all cases the yields were poor.

With the availability of powerful reducing agents such as lithium aluminum hydride, Karrer, Portmann, and Suter (18, 19) have used them for the reduction of amino acid esters and have obtained yields between 50% and 85% in the corresponding amino alcohols. Dornow, Messwarb, and Frey (10) have reduced amino acid esters with lithium aluminum hydride in boiling tetrahydrofuran. Fromageot and his collaborators (12) have applied the same reduction to esterified proteins and to different amino acid esters in order to determine the chromatographic behavior of the amino alcohols thus obtained.

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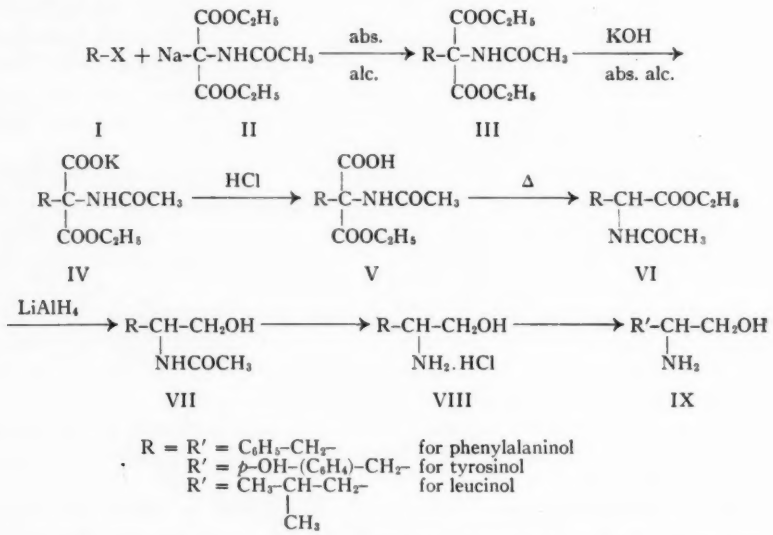
Finally in 1952, Vogl and Pöhm (24) have reduced directly the free amino acids, in suspension in boiling tetrahydrofuran, to the amino alcohols with yields of 75% to 85%. The quantities used in this case were less than one gram.

If small quantities of optically active amino alcohols are required, they can be obtained by simple procedures from L-amino acids. If, however, optical activity is not a consideration it was felt that a new direct synthesis of the amino alcohols, without the use of the amino acids, would be more economical.

Ethyl acetamidomalonate has been widely used in the synthesis of amino acids (1, 2, 22), and it has become the starting material in one of the classical methods in that field. It consists in condensing an alkyl halide with the ester and degrading by hydrolysis the resulting compound to the desired amino acid.



In order to obtain amino alcohols by modifying this method, it was necessary to proceed stepwise in the hydrolysis of the condensation product of ethyl acetamido malonate (II) with the alkyl halide (I) (the average yield in this condensation is 80%). The di-ester (III) was monosaponified in absolute alcohol with slightly more than one equivalent of potassium hydroxide. The potassic salt (IV) was then acidified and the half ester (V) was isolated with yields around 80%.

By heating the *N*-acetamido half ester (V) at 150°C., decarboxylation took place smoothly and the resulting *N*-acetamido ethyl ester (VI) was recrystallized from ligroin (yield 90%). It was reduced in the cold with lithium aluminum hydride in absolute ether or tetrahydrofuran to give the *N*-acetamido alcohol (VII).

Reduction was always carried out in the cold in order to minimize reduction at the *N*-acetamido link (8).

In the case of *o*-methyl-D,L-tyrosinol, the *N*-acetamido derivative (VII) has been isolated from ether. But the other *N*-acetamido alcohols, being liquids, were directly hydrolyzed to give the corresponding 2-amino alcohol hydrochlorides (VIII) with 70–80% yield.

The free amino alcohols (IX), except D,L-tyrosinol, were obtained by passing the hydrochloride solutions through a column of Permutit S-1.

In the case of *o*-methyl-*N*-acetyl-D,L-tyrosinol the hydrolysis with hydrochloric acid did not split the ether link, and *o*-methyl-D,L-tyrosinol was obtained. This amino alcohol when refluxed for 12 hr. with a mixture of glacial acetic acid and hydrochloric acid did not yield any D,L-tyrosinol.

o-Methyl-tyrosinol was then refluxed with a mixture of glacial acetic acid and hydrobromic acid (48%) for 12 hr. The hydrobromide was passed through a column of Permutit S-1, but the expected free D,L-tyrosinol did not come out and was adsorbed. Elution with hydrochloric acid gave D,L-tyrosinol hydrochloride.

To obviate this adsorption on the column, D,L-tyrosinol hydrobromide was neutralized with sodium carbonate and the free amino alcohol was extracted with alcohol. Free D,L-tyrosinol (70%) was recrystallized from alcohol–ether mixture.

This general method of synthesis is presently applied in this laboratory for other amino alcohols.

Results of the physiological tests will be published elsewhere.

EXPERIMENTAL PART

2-Acetamido-2-carbethoxy-3-phenylpropionic Acid

To a solution of 30.7 gm. (0.1 mole) of ethyl 2-acetamido-2-carbethoxy-3-phenylpropionate (prepared by the method of Albertson and Archer) (2) in 100 ml. of absolute alcohol was added 110 ml. of 1.0 *N* potassium hydroxide solution in absolute alcohol. The mixture was stirred and allowed to stand at room temperature overnight. It was then filtered to yield 1 gm. of inorganic salt and possibly some dipotassic salt of 2-acetamido-2-carboxy-3-phenylpropionic acid. The filtrate was concentrated *in vacuo* on the water bath, and cold water was added. Most of the residue dissolved with the exception of unreacted ethyl 2-acetamido-2-carbethoxy-3-phenylpropionate, which was filtered and dried, giving 3.0 gm. (9.8%) of the starting material.

The clear filtrate was then acidified to Congo red with hydrochloric acid. The heavy precipitate thus formed was allowed to solidify in the cold, filtered, and dried. The yield of 2-acetamido-2-carbethoxy-3-phenylpropionic acid was 23.8 gm. (85.4%). Melting point: 129–130°C.* with evolution of gas. Calc. for $C_{14}H_{17}O_5N$: N, 5.02%. Found: N, 5.04%.

Ethyl 2-Acetamido-3-phenylpropionate

In a dried flask connected to a vacuum pump there were placed 19.5 gm. of 2-acetamido-2-carbethoxy-3-phenylpropionic acid. The flask was immersed

**Melting points are not corrected.*

in a metal bath at 150°C. for 15 min. and decarboxylation took place vigorously. The resulting oil was recrystallized from ligroin yielding 14.5 gm. (88.3%) of ethyl 2-acetamido-3-phenylpropionate. Melting point: 69–70°C. Calc. for $C_{13}H_{17}O_3N$: N, 5.96%. Found: N, 5.98%.

N-Acetyl- D,L-*phenylalaninol*

In an Erlenmyer surrounded with ice and containing 12.15 gm. of ethyl 2-acetamido-3-phenylpropionate in solution in absolute ether, there were added drop by drop, by means of a separatory funnel fitted with a calcium chloride tube, 3.0 gm. of lithium aluminum hydride in 100 ml. of absolute ether. During the addition and for two more hours, the mixture was shaken with a magnetic stirrer. Excess of lithium aluminum hydride was decomposed with small lumps of ice until there was no more evolution of gas. The mixture was heated and filtered on a Büchner funnel. The inorganic solid was extracted twice with boiling alcohol and the combined extracts were evaporated *in vacuo*. The oily residue weighing 9.0 gm. did not crystallize in the cold and was then hydrolyzed.

In a subsequent run, 2.8 gm. were distilled at 160–170°C. at 6 mm. giving a viscous liquid which did not crystallize.

D,L-*Phenylalaninol Hydrochloride*

N-acetyl- D,L-phenylalaninol obtained from the preceding reduction was refluxed for three hours with 100 ml. of 2 *N* hydrochloric acid. The solution was then evaporated *in vacuo*, dissolved in water, and purified with Norit. The clear solution was again evaporated *in vacuo* and the residue was redissolved in 30 ml. of boiling alcohol. Ether was added until turbidity appeared and D,L-phenylalaninol hydrochloride crystallized out rapidly. It was collected by filtration and dried, yielding 8.1 gm. (81%) of pure product. Melting point: 154–155°C. Karrer and collaborators (17) gave a melting point of 128°C. for L-phenylalaninol hydrochloride. Calc. for $C_9H_{14}ONCl$: N, 7.48%, Cl, 18.92%. Found: N, 7.57%, Cl, 18.90%.

D,L-*Phenylalaninol*

D,L-Phenylalaninol hydrochloride (7.01 gm.) was dissolved in 50 ml. of distilled water. The solution was passed through a column of Permutit S-1. The resulting solution was free from chloride ions and had a pH above 11.0. It was evaporated to dryness and dissolved in a minimum of boiling alcohol. Ether was then added until faintly turbid and upon cooling, 4.7 gm. (83%) of pure D,L-phenylalaninol was obtained. Melting point: 73–75°C. An analytical sample, recrystallized from ether, had a melting point of 75–77°C. Litt.: 67–68°C. (18,21). The R_f value in water–phenol solution is 0.74. Calc. for $C_9H_{13}ON$: N, 9.28%. Found: N, 9.30%.

D,L-*Phenylalaninol Acid Oxalate*

D,L-Phenylalaninol (0.6 gm.) was dissolved in 5 ml. of ethanol and 3.0 gm. of oxalic acid in solution in 5 ml. of ethanol were added. The mixture was warmed a little and chilled with some scratching. D,L-Phenylalaninol

acid oxalate thus obtained weighed 0.95 gm. (98%). Melting point: 176°C. Calc. for $C_{11}H_{15}O_5N$: N, 5.81%. Found: N, 5.93%.

D,L-Phenylalaninol Neutral Oxalate

A solution of 0.2 gm. of D,L-phenylalaninol in 5 ml. of ethanol was slowly neutralized by the addition of 1.0 gm. of oxalic acid in 5 ml. of water until phenolphthalein was discolored. Upon cooling, the neutral oxalate crystallized. It was recrystallized in dilute alcohol. Yield: 2.2 gm. (81%). Melting point: 225°C. Calc. for $C_{20}H_{28}O_6N_2$: N, 7.14%. Found N, 7.20%.

N-Benzoyl-D,L-phenylalaninol

A solution containing 0.8 gm. of sodium hydroxide and 1.87 gm. of D,L-phenylalaninol hydrochloride was vigorously shaken with 1.16 ml. of benzoyl chloride. After a few minutes, the solid was filtered and dried. It was recrystallized from water and alcohol, yielding 1.7 gm. (69%) of *N*-benzoyl-D,L-phenylalaninol. Melting point: 140–142°C. Calc. for $C_{16}H_{17}O_2N$: N, 5.50%. Found: N, 5.61%.

A small quantity of another product, possibly *N*-benzoyl-*O*-benzoyl-D,L-phenylalaninol, was also collected. Melting point: 150–152°C. Calc. for $C_{23}H_{21}O_3N$: N, 3.91%. Found: N, 3.95%.

Ethyl 2-Acetamido-2-carbethoxy-3-(p-methoxyphenyl)propionate

To a stirred, boiling solution of 43.4 gm. of ethyl acetamidomalonate and 4.6 gm. of sodium in 200 ml. of absolute ethanol was added 33.0 gm. of anisyl chloride. The anisyl chloride was prepared with 85% yield from thionyl chloride, anisyl alcohol, and dimethylaniline in chloroform according to Gaudry's method (13). Refluxing was continued for four hours after which the sodium chloride was filtered. The resulting alcoholic solution was concentrated *in vacuo* and 500 ml. of ether added. Upon cooling, 53.8 gm. (80%) of crystalline ethyl 2-acetamido-2-carbethoxy-3-(*p*-methoxyphenyl)propionate were obtained. The melting point was 101°C. Litt.: 96°C. (5). Calc. for $C_{17}H_{23}O_6N$: N, 4.16%. Found: N, 4.12%.

2-Acetamido-2-carbethoxy-3-(p-methoxyphenyl)propionic Acid

In a flask containing 370 ml. of 0.3 *N* alcoholic potassium hydroxide was added 33.7 gm. (0.1 mole) of ethyl 2-acetamido-2-carbethoxy-3-(*p*-methoxyphenyl)propionate. The mixture was agitated with a magnetic stirrer overnight. It was then treated according to the procedure described for 2-acetamido-2-carbethoxy-3-phenylpropionic acid. Yield of 2-acetamido-2-carbethoxy-3-(*p*-methoxyphenyl) propionic acid: 27.2 gm. (88%). Melting point: 132–133°C. with evolution of gas. It was recrystallized by solution in a minimum of boiling alcohol which was then rapidly poured over cracked ice. Calc. for $C_{15}H_{19}O_6N$: N, 4.54%. Found: N, 4.59%.

If the amount of added potassium hydroxide is less than the theoretical quantity, unreacted starting material is recovered without any loss or contamination. If the alcohol contains an appreciable amount of water, some spontaneous decarboxylation might occur during or before acidification, in

which case the decarboxylated ester is recovered by ethereal extraction of the acidified water.

Ethyl 3-(p-Methoxyphenyl)-2-acetamido Propionate

The decarboxylation was carried out in the same way as for ethyl 2-acetamido-3-phenylpropionate. The 2-acetamido-2-carbethoxy-3-(*p*-methoxyphenyl)-propionic acid (21.3 gm.) was decarboxylated to yield 17.4 gm. (95%) of 3-(*p*-methoxyphenyl)-2-acetamido propionate. When recrystallized from ligroin, it had a melting point of 72–73°C. Calc. for $C_{14}H_{19}O_4N$: N, 5.29%. Found, 5.30%.

o-Methyl-N-acetyl- D,L*-tyrosinol*

The reduction was carried out as for *N*-acetyl- D,L-phenylalaninol. Ethyl 3-(*p*-methoxyphenyl)-2-acetamido propionate (15.5 gm.) was reduced in cold absolute ether with 3.0 gm. of lithium aluminum hydride. The *o*-methyl-*N*-acetyl- D,L-tyrosinol thus obtained weighed 13.1 gm. (88%). An analytical sample was recrystallized from ether and had a melting point of 110–111°C. Calc. for $C_{12}H_{17}O_3N$: N, 6.28%. Found: N, 6.28%.

o-Methyl- D,L*-tyrosinol Hydrochloride*

o-Methyl-*N*-acetyl- D,L-tyrosinol (9.5 gm.) was hydrolyzed as described for D,L-phenylalaninol hydrochloride. *o*-Methyl- D,L-tyrosinol hydrochloride crystallized from alcohol–ether mixture. It was collected and dried. Yield: 7.92 gm. (85.6%). Melting point: 195–197°C. Calc. for $C_{10}H_{16}O_2NCl$, 16.3%. N, 6.47%. Found: Cl, 16.2%. N, 6.44%.

o-Methyl- D,L*-tyrosinol*

A solution containing 3.0 gm. of *o*-methyl- D,L-tyrosinol hydrochloride in 50 ml. of water was passed through a column of Permutit S-1. The resulting solution, which was free from chloride ions, was evaporated to dryness. The residue was recrystallized from ether yielding 2.1 gm. (84%) of *o*-methyl- D,L-tyrosinol. Melting point: 80–82°C. Calc. for $C_{10}H_{15}O_2N$: N, 7.74%. Found: N, 7.83%.

o-Methyl- D,L*-tyrosinol Neutral Oxalate*

The *o*-methyl- D,L-tyrosinol neutral oxalate, recrystallized from water and alcohol, had a melting point of 220°C. Calc. for $C_{22}H_{32}O_8N_2$: N, 6.20%. Found: N, 6.21%.

o-Methyl- D,L*-tyrosinol Acid Oxalate*

o-Methyl- D,L-tyrosinol acid oxalate recrystallized from alcohol had a melting point of 160°C. Calc. for $C_{12}H_{17}O_6N$: N, 5.17%. Found: N, 5.10%.

D,L*-Tyrosinol*

A solution of 1.18 gm. of *o*-methyl- D,L-tyrosinol in 50 ml. of glacial acetic acid and 50 ml. of hydrochloric acid was refluxed for 12 hr. The starting material was recovered. It did not give the Millon test for phenolic group.

A solution containing 6.35 gm. of *o*-methyl- D,L-tyrosinol in 50 ml. of glacial acetic acid and 50 ml. of 48% hydrobromic acid was refluxed for 12 hr. The mixture was evaporated to dryness, and dissolved in water.

Half of the solution was passed through a column of Permutit S-1. The filtrate did not give the ninhydrin or the Millon tests. Alcohol was then passed through the column and this alcoholic filtrate was also negative to the previous tests. Both filtrates were combined and evaporated to dryness. No residue was obtained. 2 *N* Hydrochloric acid was then passed through the column and the acid eluate was evaporated to dryness yielding 2.6 gm. of a viscous oil which gave a positive ninhydrin and Millon test.

The other half of the solution was evaporated to dryness and then dissolved in a minimum of water. A solution of sodium carbonate was then added until pH 9.0 was obtained. The mixture was evaporated to dryness and the residue was dissolved in 20 ml. of boiling alcohol. Insoluble salts were filtered and the alcoholic filtrate was again evaporated. The residue was recrystallized by solution in boiling alcohol and addition of ether until turbidity appeared. D,L-Tyrosinol crystallized yielding 2.0 gm. (70%). Melting point: 128–129°C. Litt.: 94°C. for L-tyrosinol (11, 18). Calc. for $C_9H_{13}O_2N$: N, 5.40%. Found: N, 5.50%.

D,L-Tyrosinol Hydrochloride

D,L-Tyrosinol was neutralized by addition of hydrochloric acid. The D,L-tyrosinol hydrochloride thus obtained was recrystallized from alcohol–ether mixture. Melting point: 186°C. Calc. for $C_9H_{14}O_2NCl$: N, 6.88%. Cl, 17.42%. Found: N, 6.78%. Cl, 17.32%.

D,L-Tyrosinol Neutral Oxalate

D,L-Tyrosinol neutral oxalate, recrystallized from methanol, had a melting point of 231°C.

D,L-Tyrosinol Acid Oxalate

The melting point of D,L-tyrosinol acid oxalate recrystallized from alcohol–ether mixture was 166°C.

2-Acetamido-2-carbethoxy-4-methyl-pentanoic Acid

A solution containing 27.3 gm. of ethyl 2-acetamido-2-carbethoxy-4-methylpentanoate (prepared according to the method of Albertson and Archer (2)) in 370 ml. of 0.3 *N* alcoholic potassium hydroxide was agitated overnight in a closed flask by means of a magnetic stirrer.

The solution was then treated as described for 2-acetamido-2-carbethoxy-3-phenyl propionic acid. Yield of 2-acetamido-2-carbethoxy-4-methylpentanoic acid: 19.6 gm. (80%).

An analytical sample was recrystallized from water. Melting point: 130–132°C. with evolution of gas. Calc. for $C_{11}H_{19}O_5N$: N, 5.72%. Found: 5.73%.

Ethyl 2-Acetamido-4-methyl-pentanoate

In a flask connected to a vacuum pump, 10.8 gm. of 2-acetamido-2-carbethoxy-4-methyl-pentanoic acid were heated at 150°C. *in vacuo* for 15 min. Decarboxylation proceeded smoothly and the resulting oil was distilled; 8.0 gm. (90.3%) of ethyl 2-acetamido-4-methylpentanoate passed at 148–

152°C. at 7 mm. The clear oil did not crystallize after a prolonged period in the cold.

N-acetyl D,L-*Leucinol*

The reduction was carried out as for *N*-acetyl-D,L-phenylalaninol. Ethyl 2-acetamido-4-methyl-pentanoate (8.0 gm.) was reduced with 3.0 gm. of lithium aluminum hydride. *N*-acetyl-D,L-leucinol was isolated as an oil, weighing 4.6 gm. (72.8%), and distilling at 172–175°C. at 9 mm. It did not crystallize in the cold.

D,L-*Leucinol Hydrochloride*

A solution containing 4.6 gm. of *N*-acetyl-D,L-leucinol was treated according to the procedure described for D,L-phenylalaninol hydrochloride. The recrystallized D,L-leucinol hydrochloride weighing 3.9 gm. (88%) had a melting point of 161–162°C. Calc. for $C_6H_{16}ONCl$: N, 9.13%. Cl, 23.8%. Found: N, 9.22%. Cl, 24.3%.

D,L-*Leucinol Neutral Oxalate*

A solution of free D,L-leucinol was obtained by passing 1.0 gm. of D,L-leucinol hydrochloride through a column of Permutit S-1. From this solution D,L-leucinol neutral oxalate was prepared. Recrystallized from alcohol, it had a melting point of 206°C. Calc. for $C_{14}H_{32}O_6N_2$: N, 8.64%. Found: N, 8.61%.

D,L-*Leucinol Acid Oxalate*

In the same way, D,L-leucinol acid oxalate was prepared. Melting point: 179°C. Calc. for $C_8H_{17}O_5N$: N, 6.77%. Found: N, 6.70%.

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THE PHOTOLYSIS OF MERCURY DIMETHYL WITH 3-ETHYL PENTANE[1]

By Richard E. Rebbert[2] and E. W. R. Steacie

ABSTRACT

Mercury dimethyl was photolyzed in the presence of 3-ethyl pentane in the temperature range from 76°C. to 238°C. The activation energy for the reaction

$$CH_3 + C_7H_{16} \rightarrow CH_4 + C_7H_{15}$$

was found to be 6.8 ± 0.3 kcal. per mole.

INTRODUCTION

In a previous paper (1) the activation energies and the rate constants for a number of reactions of the type

$$CH_3 + RH \rightarrow CH_4 + R$$

were determined. To these we can now add the present hydrocarbon, 3-ethyl pentane. So far as the authors know, this reaction has not been investigated before by any method.

EXPERIMENTAL

The experimental set-up was exactly the same as that reported previously (1). No filters were used. The mercury dimethyl was the same as before and was better than 99% pure. The 3-ethyl pentane was a standard sample from the American Petroleum Institute, 225–5S. The stated impurity was 0.13 ±0.03 mole per cent.

Results

The results are given in Table I and are plotted in Fig. 1. The method of calculating the results is exactly the same as before.

TABLE I

The photolysis of mercury dimethyl with 3-ethyl pentane

Temp., °K.	Pressure of $Hg(CH_3)_2$ (mm.)	Pressure of $CH(C_2H_5)_3$ (mm.)	Time (sec.)	Products (μM.)		$k_1/k_2^{\frac{1}{2}} \times 10^{13}$
				CH_4	C_2H_6	
349	29.8	34.6	3600	1.23	15.66	3.47
378	38.0	32.0	2700	1.99	13.65	6.77
394	40.2	33.1	2700	3.06	13.91	10.2
420	42.0	35.1	1800	3.40	9.13	16.6
446	44.2	36.9	1500	4.65	7.02	27.6
470	48.0	39.3	1200	5.58	5.23	40.7
497	50.1	41.1	1200	8.21	5.35	56.0
511	50.1	41.1	900	8.24	3.84	81.3

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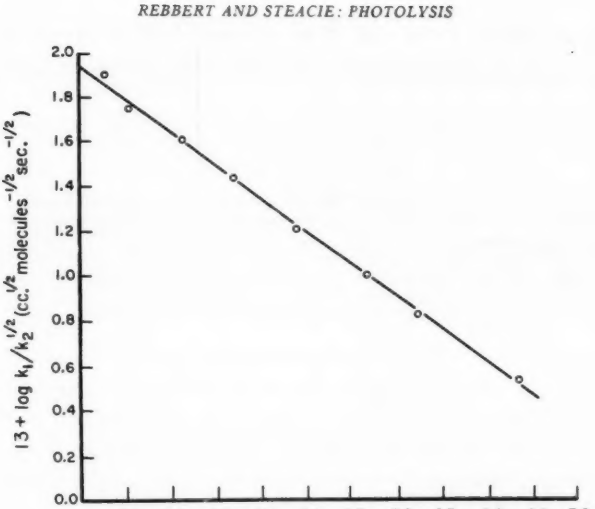

FIG. 1. Arrhenius plot for reaction of methyl radicals with 3-ethyl pentane.

Discussion

The main steps of the over-all reaction are the following

$$Hg(CH_3)_2 + h\nu \rightarrow Hg + 2CH_3 \qquad [0]$$

$$CH_3 + Hg(CH_3)_2 \rightarrow CH_4 + CH_3HgCH_2. \qquad [1']$$

$$CH_3 + C_7H_{16} \rightarrow CH_4 + C_7H_{15} \qquad [1]$$

$$2CH_3 \rightarrow C_2H_6. \qquad [2]$$

We assume that methane and ethane are not formed by any other reaction than those postulated above. The fate of CH_3HgCH_2 and C_7H_{15} may be dimerization, reaction with each other, or reaction with a methyl radical.

The above mechanism leads to an activation energy for reaction [1] of 6.8 kcal. per mole. The best line through all points has been found by the method of least squares to be $13 + \log k_1/k_2^{\frac{1}{2}} = 4.76 - (1478/T)$. If P_2 is assumed to be unity, the steric factor, P_1, is 1.2×10^{-4}.

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THE HYDROLYSIS OF THE CONDENSED PHOSPHATES

I. SODIUM PYROPHOSPHATE AND SODIUM TRIPHOSPHATE[1]

By Joan Pedley Crowther and A. E. R. Westman

ABSTRACT

The rates of hydrolysis of sodium pyrophosphate and triphosphate in solution have been measured at 65.5°C. over the pH range 2.0 to 12.0 and the phosphorus concentration range 0.10 to 0.25 atomic weights per liter. The reactions were found to be first order providing a constant concentration of hydrogen ion was maintained in the reaction flask. Both reactions are acid catalyzed but only the hydrolysis of triphosphate was found to be base catalyzed. Pyrophosphate and triphosphate apparently hydrolyze independently of each other.

INTRODUCTION

The hydrolysis of condensed phosphates is of interest commercially because the complexing properties of these materials are not possessed by the ultimate reversion product, orthophosphate. It is not surprising then that many workers have investigated the hydrolysis of sodium pyrophosphate and triphosphate. The value of the work, however, is frequently diminished by the failure to control the hydrogen ion concentration during the course of the reaction (the hydrolysis produces weaker acids resulting in an increase in pH), and because good analytical procedures were not available.

The inadequacies of analytical methods for phosphates are not so apparent in the studies of the hydrolysis of pyrophosphate since pyrophosphate reverts directly to orthophosphate, and orthophosphate can be determined in the presence of other phosphates. By such a procedure Green (3) and Muus (6) investigated the hydrolysis of pyrophosphate.

When the investigations of the hydrolysis of triphosphate are examined, however, it becomes very obvious that these studies have been handicapped by poor analytical procedures. The fact that one mole of triphosphate anion reverts to one mole of pyrophosphate and one mole of orthophosphate anion was established by Bell (1) as recently as 1947. Bell (1) employed an analytical method (2) which he had developed and which permitted the direct determination of triphosphate. However, most workers studied the hydrolysis of the triphosphate anion indirectly. Thus Green (3) followed the course of the reaction by measuring the amount of orthophosphate formed. Morgan and Swoope (5) could not measure directly the triphosphate, and thus could not study effectively the hydrolysis of commercial sodium 'tetraphosphate'. Similarly Watzel (8) could not directly determine the triphosphate.

The analytical method (9) used by the authors permits a direct determination of each component with adequate accuracy and provides qualitative as well as quantitative information on the system.

The study of the hydrolysis of pyrophosphate and triphosphate has also been complicated by the failure to recognize the importance of the hydrogen ion

[1] *Manuscript received September 28, 1953.*
Contribution from the Department of Chemistry, Ontario Research Foundation, Toronto, Ontario.

concentration. Even when the initial hydrogen ion concentration and its subsequent changes are measured (the procedure followed by Muus(6)), the worker must deal with the additional variable, the hydrogen ion concentration. Green (3) avoided this by measuring the rate of reaction at a series of hydrogen ion concentrations. From his data the dependence of the rate of hydrolysis on the hydrogen ion and phosphate ion concentration could be evaluated. In the present investigation the experimental design was similar to that of Green (3). However, a more efficient analytical procedure (9) was employed and the effect of the hydrogen ion concentration was investigated over a wider range.

EXPERIMENTAL

Materials

The preparation of pure sodium triphosphate is very difficult, and in the present investigation a commercial grade was used. The sodium pyrophosphate used was initially believed to be pure. When the analytical procedure was improved, however, it was found that one per cent of the phosphorus was present as orthophosphate.

A stock solution of sodium pyrophosphate (0.245 atomic weights phosphorus per liter) was used in the experiments. This concentration was selected because it was suitable for the analysis.

The sodium triphosphate was 87.3 mole per cent sodium triphosphate and 12.7 mole per cent sodium pyrophosphate. The concentration of the stock solution used was 0.190 atomic weights phosphorus per liter—this value being suitable for analysis.

Procedure

Samples (100 ml.) prepared from the stock phosphate solutions were placed in a long narrow tube fitted with a stirring rod and a reflux condenser. The solutions were heated by suspending the tube in a water bath maintained at 65.5 ± 0.1°C. This temperature was selected so that the experimental results could be compared with Green's (3) results obtained at 150°F. Since the pH values of sodium pyrophosphate and triphosphate solutions are approximately 9.5, and since their rates of hydrolysis in this region are very small, it was assumed that the solutions did not undergo reversion while they were being heated to 65.5°C. To adjust the phosphate solution approximately to the desired pH value, either 10 *N* sulphuric acid or sodium hydroxide pellets was added. The time at which either was added was considered to be the time at which the hydrolysis reaction began. The pH was finally adjusted and maintained within ±0.1 units of the desired value by adding known volumes of *N*/10 ammonium hydroxide and *N*/10 sulphuric acid. The pH values were measured with a glass electrode and a Leeds and Northrup pH meter (Cat. No. 7663) which had a sensitivity of 0.02 pH units. After each pH measurement the sample was returned to the reaction flask. At measured time intervals samples were withdrawn and spotted on the filter paper chromatogram within one minute. Further hydrolysis during the analysis was negligible.

Analysis

The analyses were made using a procedure (9) developed in this laboratory. This method involves the separation of the phosphate components by filter paper chromatography and the subsequent colorimetric determination of the phosphorus content of each component. The accuracy of the method is an absolute figure ± 1.0%.

Data

The hydrolysis of sodium pyrophosphate in solution was studied at 65.5°C. over the pH range 2.0 to 10.8. At pH 2.0, the solution concentrations were 0.082, 0.123, and 0.245 atomic weights phosphorus per liter. In the remainder of the experiments the solution concentration was 0.245 atomic weights phosphorus per liter. The compositions of these solutions, after various time periods, are given in Table I.

TABLE I
HYDROLYSIS OF PYROPHOSPHATE

pH	Concentration (moles phosphorus per liter)	Reaction time (min.)	% Distribution of phosphorus		k (First order rate constant) (min.$^{-1}$)	Average k (min.$^{-1}$)
			Pyro-phosphate	Ortho-phosphate		
—	—	0	99.0	1.0		
2.0	0.245	60	95.6	4.3	5.76×10^{-4}	
		120	92.4	7.6	5.76×10^{-4}	
		180	90.1	9.9	5.25×10^{-4}	
		240	87.0	13.0	5.37×10^{-4}	5.53×10^{-4}
2.0	0.123	120	92.2	7.8	5.76×10^{-4}	
		180	90.0	10.0	5.12×10^{-4}	
		240	87.4	12.6	5.19×10^{-4}	5.36×10^{-4}
2.0	0.082	120	92.4	7.7	5.76×10^{-4}	
		240	86.4	13.6	5.66×10^{-4}	
		360	82.8	17.2	4.99×10^{-4}	
		420	80.0	20.0	5.04×10^{-4}	5.36×10^{-4}
3.0	0.245	242	92.1	7.9	2.94×10^{-4}	
		425	86.1	13.9	3.25×10^{-4}	
		1344	61.0	39.0	3.59×10^{-4}	
		1655	55.0	45.0	3.54×10^{-4}	
		1840	54.4	45.6	3.25×10^{-4}	3.31×10^{-4}
6.0	0.245	128	96.7	3.3	1.45×10^{-4}	
		260	95.0	5.0	1.24×10^{-4}	
		368	95.0	5.0	0.88×10^{-4}	
		418	91.4	8.6	1.43×10^{-4}	1.25×10^{-4}
9.3	0.245	425	98.6	1.4	1.1×10^{-5}	
		1380	97.5	2.5	1.0×10^{-5}	
		1865	97.4	2.6	0.9×10^{-5}	1.0×10^{-5}
10.9	0.245	2820	98.4	1.6	1.6×10^{-6}	
		3250	97.4	2.6	4.9×10^{-6}	
		4250	95.5	4.5	7.9×10^{-6}	4.8×10^{-6}

Similarly the hydrolysis of sodium triphosphate in solution was studied at 65.5°C. over the pH range 2.0 to 12.0. At pH 2.0, the solution concentrations

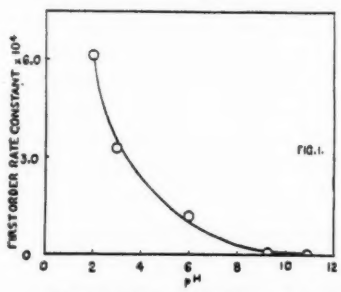
were 0.190 and 0.0951 atomic weights phosphorus per liter. In the remainder of the experiments the solution concentration was 0.0951 atomic weights phosphorus per liter. The compositions of these solutions after various time periods are given in Table II.

TABLE II
HYDROLYSIS OF TRIPHOSPHATE

pH	Concentration (moles phosphorus per liter)	Reaction time (min.)	% Distribution of phosphorus			k (First order rate constant) (min.$^{-1}$)	Average k (min.$^{-1}$)
			Tri-phosphate	Pyro-phosphate	Ortho-phosphate		
—	—	0	91.2	8.8	0		
2.0	0.0951	60	77.9	17.0	5.0	2.58×10^{-3}	
		135	70.5	20.4	9.2	1.89×10^{-3}	
		295	49.7	31.6	18.7	2.04×10^{-3}	
		425	33.8	40.0	26.3	2.32×10^{-3}	
		1395	4.7	35.1	60.3	2.11×10^{-3}	2.19×10^{-3}
2.0	0.190	96	74.6	18.0	7.4	2.06×10^{-3}	
		225	47.8	27.7	14.5	2.02×10^{-3}	
		345	44.6	35.5	19.9	2.06×10^{-3}	2.05×10^{-3}
3.0	0.0951	238	73.5	20.6	6.0	9.08×10^{-4}	
		400	61.5	25.4	13.0	9.76×10^{-4}	
		1360	24.9	42.5	32.6	9.52×10^{-4}	9.4×10^{-4}
5.0	0.0951	120	87.7	11.0	1.3	3.26×10^{-4}	
		240	85.2	12.6	2.3	2.78×10^{-4}	
		360	83.6	13.9	2.4	2.36×10^{-4}	
		420	79.8	14.6	5.7	3.17×10^{-4}	2.89×10^{-4}
9.3	0.0951	317	90.8	9.2	0	1.1×10^{-3}	
		2818	86.6	12.2	1.2	1.8×10^{-3}	
		3202	86.4	12.4	1.2	1.7×10^{-3}	1.5×10^{-3}
12.0	0.0951	1360	76.5	17.5	6.0	1.29×10^{-4}	
		1635	71.6	19.9	8.7	1.51×10^{-4}	
		1820	70.4	20.6	8.9	1.43×10^{-4}	1.41×10^{-4}

Calculations

When the data in Tables I and II were substituted in the general equation for a first order reaction, the rate constants (calculated for each hydrogen ion



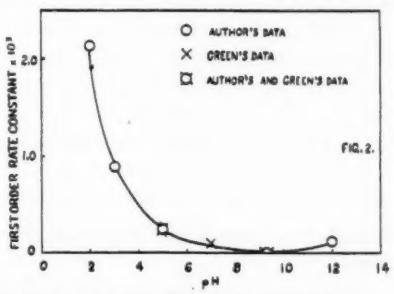


FIG. 1. Effect of pH on rate of hydrolysis of sodium pyrophosphate in solution.
FIG. 2. Effect of pH on rate of hydrolysis of sodium triphosphate in solution.

concentration studied) were found to be constant within the experimental accuracy, even when the initial phosphorus concentration was varied. The values of these rate constants, for the hydrolysis of pyrophosphate and triphosphate, are included in Tables I and II respectively. Figs. 1 and 2 are the curves obtained by plotting the first order rate constants, for pyrophosphate and triphosphate solutions respectively, against the pH of the solutions.

DISCUSSION

Order of Reaction

The above results are in agreement with Muus' (6) theory that the hydrolysis of the pyrophosphate anion is a first order reaction at constant hydrogen ion concentration. Muus (6) was unable to establish this theory experimentally since he did not maintain a constant hydrogen ion concentration in his experimental work, and only studied the reaction over the pH range 0.91 to 1.3, but he deduced the concept from his results.

On the other hand, Green (3) found that the first order rate constants for the hydrolysis of the triphosphate anion depended upon its initial concentration. Green's (3) first order rate constants for a 0.005% sodium triphosphate solution are included in Fig. 2. The concentration of the sodium triphosphate solution, which we studied, was 1.17%. Different analytical procedures were used, and Green (3) determined the rate of reaction by the amount of orthophosphate formed. Under these circumstances, the agreement between the two sets of data is quite acceptable. However, Green (3) found that the rate constants which he determined for a 0.0005% sodium triphosphate solution disagreed with those determined for the 0.005% solution. Possibly in very dilute solutions the reaction may not be first order with respect to the triphosphate concentration, but certainly it appears to be so over the concentration range 0.005 to 1.17%.

In conclusion, the results indicate that the hydrolysis of triphosphate anion or pyrophosphate anion is first order at constant hydrogen ion concentration over the pH range 2.0 to 12.0.

Acid–Base Catalysis

An examination of Figs. 1 and 2 indicates that the hydrolyses of pyrophosphate and triphosphate in solution are both acid catalyzed while only the triphosphate hydrolysis is base catalyzed over the pH range studied. Triphosphate solutions are most stable in the pH range 9 to 10, and this finding is in accordance with Watzel's (8) results. Van Wazer, Griffith, and McCullough (7) have suggested that this apparent base catalysis of triphosphate is due to the formation of complexes with sodium ion. The discussion of the effect of the hydrogen ion concentration on the hydrolysis of pyrophosphate solutions has been reserved for a second paper by McGilvery and Crowther (4).

Hydrolysis of Solutions Containing Both Pyrophosphate and Triphosphate Anions

Whether or not triphosphate and pyrophosphate hydrolyze independently of each other can be ascertained from an examination of the course of hydroly-

sis of sodium triphosphate and the corresponding first order rate constants for pyrophosphate and triphosphate. That is, the proportion of pyrophosphate was measured at various times as the hydrolysis of triphosphate solutions proceeded, and this proportion can be calculated from the following relationship if pyrophosphate and triphosphate hydrolyze independently:

$$P = b\left(\frac{a-x}{a}\right)^{k_2/k_1} + \frac{2}{3}\left(\frac{k_1 a}{k_2 - k_1}\right)\left\{\left(\frac{a-x}{a}\right) - \left(\frac{a-x}{a}\right)^{k_2/k_1}\right\}$$

where P is the percentage phosphorus as pyrophosphate at time t

b is the initial percentage phosphorus as pyrophosphate

x is the percentage phosphorus as triphosphate which has decomposed at time t

a is the initial percentage phosphorus as triphosphate

k_1 is the first order rate constant for the hydrolysis of triphosphate anion at a specified hydrogen ion concentration

k_2 is the first order rate constant for the hydrolysis of pyrophosphate anion at the same hydrogen ion concentration

The calculated and measured percentages of phosphorus as pyrophosphate are shown in Table III. These values are in satisfactory agreement indicating that pyrophosphate and triphosphate hydrolyze independently.

TABLE III

TEST OF INDEPENDENCE OF THE HYDROLYSIS OF TRIPHOSPHATE AND PYROPHOSPHATE

pH	Reaction time	% Phosphorus as pyrophosphate (measured)	% Phosphorus as pyrophosphate (calculated)
—	0	8.8	—
2.0	60	17.0	17.1
	96	18.0	19.1
	135	20.4	21.6
	225	27.7	28.8
	295	31.6	33.0
	345	35.5	35.4
	425	40.0	39.9
	1395	35.1	38.2
3.0	238	20.6	19.5
	400	25.4	26.1
	1360	42.5	39.4
5.0	120	11.0	11.1
	240	12.6	12.6
	360	13.9	13.6
	420	14.6	15.5
9.3	317	9.2	9.0
	2818	12.2	11.5
	3202	12.4	11.6
12.0	1360	17.5	18.5
	1635	19.9	21.8
	1820	20.6	22.6

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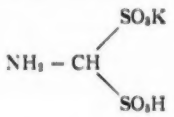
NOTE

MONO- AND DIPOTASSIUM AMINOMETHIONATE

By R. A. B. Bannard[1] and J. H. Ross

In 1895, von Pechmann and Manck (3) isolated monopotassium aminomethionate (I) and dipotassium aminomethionate (II) from the solution which resulted on addition of crystalline potassium cyanide to a potassium bisulphite solution. When prepared according to the directions given by these workers (3) monopotassium aminomethionate was obtained in yields of approximately 40% rather than the 58–72% range claimed, and a more reliable method of preparation was sought.



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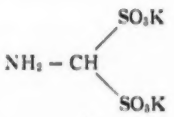

II

Recently, Arnold and Perry (1, 2) have patented a process for the preparation of sodium and potassium salts of aminomethionic acid. In their method, a solution of an alkali-metal cyanide is added to an alkali-metal bisulphite solution at 40 to 45°C. over a prolonged period, with addition of sulphur dioxide to neutralize the alkalinity developed by the reaction and to precipitate the salts at the conclusion of the process. Arnold and Perry (1, 2) obtained a 30% yield of the disubstituted salts or a 40% yield of the monosubstituted salts, and found that the yield of the latter increased to 80% on addition of fresh cyanide to the liquor after the monosubstituted salt was removed.

Not wishing to resort to a recycling operation for laboratory preparation we have modified Arnold and Perry's (1, 2) method by (i) using more concentrated bisulphite solutions (3.5 molar), (ii) increasing the reaction temperature to 55–80°C., (iii) holding the pH of the solution at 7.85 during heating, and (iv) precipitating monopotassium aminomethionate at pH 2.5. These modifications have increased the yield of monopotassium aminomethionate to 68.0% and shortened the reaction time from 12 hr. to 35 min.

Dipotassium aminomethionate was prepared in 68.5% yield simply by neutralization of the monopotassium salt with potassium hydroxide in aqueous solution.

EXPERIMENTAL

Monopotassium Aminomethionate

A 400-ml. Berzelius beaker was equipped with glass and calomel electrodes, a thermometer, mechanical stirrer, burette, and fritted-glass gas disperser

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connected to a sulphur dioxide cylinder. A potassium bisulphite – sulphite solution was prepared from 14.6 gm. (equivalent to 0.125 mole $KHSO_3$) of potassium metabisulphite, 93.9 gm. (0.581 mole) of potassium sulphite and 140 ml. of water, and placed in the reaction vessel. The burette was filled with a solution of 23.2 gm. (0.349 mole) of potassium cyanide in 35 ml. of water. The bisulphite – sulphite solution was heated to 55°C.; simultaneous addition of cyanide solution and sulphur dioxide was begun and continued in order to maintain the pH at 7.85, while the temperature was raised at a rate of approximately 1°C. per minute until a maximum of 80°C. was reached (20 min.). At this point all the cyanide solution had been added but addition of sulphur dioxide was continued for a further 15 min. at 80°C. to hold the pH at 7.85. The solution was cooled to 20°C. over a period of 20 min. and acidified to pH 2.50 by addition of sulphur dioxide. After standing for 30 min. at 10°C. the precipitated salts were collected on a fritted-glass funnel by suction filtration and washed with two 100-ml. portions of water to remove coprecipitated potassium bisulphite. The colorless microcrystalline powder was air-dried yielding 54.4 gm. (68.0%) of pure monopotassium aminomethionate.

Dipotassium Aminomethionate

Monopotassium aminomethionate, 45.8 gm. (0.200 mole) was suspended in 100 ml. of water and the mechanically stirred suspension treated with 10% potassium hydroxide solution until a clear solution was obtained. The pH of the solution was adjusted to 8.00 by addition of concentrated hydrochloric acid. The solution was filtered, diluted to a volume of 300 ml., and allowed to stand at 0°C. for 12 hr. The colorless crystals of dipotassium aminomethionate monohydrate which separated were collected by suction-filtration and weighed 39.0 gm. (68.5%) after air-drying.

We wish to gratefully acknowledge the award of a National Research Council Studentship to one of us (R.A.B.B.) and financial assistance from the Defence Research Board of Canada.

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